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Methods of Spore Measurement in the Actinomyces. F. M. BLODGETT.

From spore measurements, alone, strains of Actinomyces, isolated from potato-scab lesions and found to be pathogenic, can be separated into numerous groups. To make the measurements an apparatus was designed that consists of a filar micrometer operated by a flexible shaft, which makes it possible to avoid disturbing the focus of the microscope during a measurement, as so easily occurs with the ordinary filar micrometer. The apparatus as built proved adequate and convenient for its purpose. A standard synthetic culture medium proved essential, as did also a rather elaborate method of sampling, if successive samples were to agree as closely as expected. The measurements did not fit a normal frequency curve, but the curves were more or less skewed. However, it was found that with an adequate sampling technique, even though the curves were skewed, the usual constants, the standard deviation, and the mean and standard error of the means were fairly reliable guides. The *chi*-square test did provide a somewhat more satisfactory method, inasmuch as it compares the frequencies in all parts of the range of sizes, and it is based on no assumption as to the shape of the frequency distribution involved.

Stimulation of Potatoes by Magnesium Bordeaux Spray. REINER BONDE.

Spraying potatoes with Bordeaux mixture prepared with a high-calcium hydrated lime has failed to stimulate plant growth and to increase yield in Aroostook County, Maine, when applied in the absence of insects and diseases. Bordeaux, prepared with a hydrated lime high (about 30%) in magnesium, has stimulated potato-plant growth and yield on Aroostook soil deficient in magnesium. Magnesium deficiency causes general stunting, chlorosis, and necrosis of the lower leaves, along the veins, which may involve the whole plant. Deficient plants sprayed with magnesium Bordeaux showed recovery in the new growth and lived longer. In one test the yield increase was 96 bushels an acre, or 343 per cent of the yield of 28 bushels secured in adjacent plots sprayed with calcium Bordeaux. On less deficient soil in the same field, the yield was increased only 15 per cent of the 253 bushels per acre secured with calcium Bordeaux. In another test the magnesium Bordeaux increased the yield 133 per cent on deficient soil and 11 per cent on better soil. It is suggested that some spray stimulation reported in the past may have been due to magnesium absorption.

Strawberry Dwarf in Massachusetts. O. C. BOYD, J. R. CHRISTIE and N. E. STEVENS.

Strawberry dwarf (*Aphelenchoides fragariae*) was first observed in Massachusetts in 1931. There were then found only traces of infection in a few beds of the Blakemore and Howard 17 (Premier) varieties. In 1932 and 1933, dwarf was more widespread, the symptoms more pronounced and the injury much greater. Losses in individual fields of the Howard 17 variety ranged up to 50 per cent of plants killed. Seasonal behavior of diseased plants is different in Massachusetts and in the south. In most of the southern States symptoms of dwarf are conspicuous in summer and the plants are nearly or quite normal during the spring. On Cape Cod dwarf symptoms are most conspicuous in the spring. Both in seasonal relations and appearance of severely diseased plants the disease, in Massachusetts, resembles "Cauliflower" of strawberries in Europe (caused by the same nematode) rather than typical "dwarf" in this country. The nematode population of diseased plants in Massachusetts is very high. One plant, examined early in May, was found to harbor 11,000 *A. fragariae*. In contrast the highest nematode population on dwarfed plants in North Carolina, examined weekly over a period of nearly 2 years, was about a thousand.

A Mosaic Disease of Bulbous Iris. PHILIP BRIERLEY and FRANK P. MCWHORTER.

The establishment of commercial Spanish and Dutch bulbous-iris plantings in America has been hindered by a destructive mosaic disease, introduced on the stocks imported from Europe. Infected plants are dwarfed, develop yellow-striped or mottled leaves and flower sheaves and blotched flowers. Investigations, 1929–1933, prove it a transmissible mosaic disease, with results as follows: Varietal and intervarietal tissue grafts (diseased tissue inserted into slits in healthy stems), 42 trials totaling 453 plants averaged 41 per cent mosaic transfer; hypodermic-needle injections into nodes, 37 trials, 477 plants, averaged 12 per cent; into internodal pith, 5 trials, 46 plants averaged 56 per cent. Inoculation by single and multiple needle and by rubbing methods has not been successful. Flower and pod cutting tests were negative, indicating little danger of accidental mechanical transfer during ordinary cultural operations. Natural transmission appears to be due to aphids. Cage tests gave the following results: *Myzus persicae*, 4 trials, 50 plants averaged 50 per cent transmission; *Illinoia solanifolii*, 14 trials, 122 plants averaged 31 per cent. Tests were negative with *Myzus pelargonii* and *M. circumflexus* and with the "bulb aphids" *Anuraphis tulipae* and *Rhopalosiphoninus tulipaella*. Transfer by aphids occurred only where the insects migrated naturally from diseased to healthy plants within the cage. All infection experiments were checked by noninoculated sister-bulb controls and nonviruliferous aphids.

Attempted Removal of Staling Substances of Fungus Cultures. J. C. CARTER.

Helminthosporium sativum and a bacterial organism designated as $9a_2$ are used to stale the medium. Fifty c.c. of nutrient broth are placed in 250 c.c. flasks and inoculated with *Helminthosporium sativum*. Two days later the same nutrient broth is inoculated with $9a_2$. The cultures are incubated for 10 days in the dark at room temperature. The staled medium is filtered through filter paper and Berkefeld filters. The filtrate is divided into 2 equal portions. Portion No. 1 is dialized in a Bradfield dializer for 45 minutes. Portion No. 2 is not dialized but is otherwise treated as is No. 1. Nutrient broth is added to the water accumulated in the + and — compartments of the dializer; pH readings are taken on all materials obtained before and after dialysis. A half of the solution obtained from each of the 3 compartments is reinoculated with *H. sativum*. The other half of the various solutions is reinoculated only after the pH value of the original broth (pH 6.86) has been restored. All solutions are sterilized before reinoculation. Sterilization does not inactivate the staling agent. Filtered broth inoculated with *H. sativum* serves as a check. Readings showing the percentage of fungus growth are taken after 6 days. Results indicate that the staling agent is attracted to the negative pole of the Bradfield dializer.

Fungi and Bacteria on Barley. J. J. CHRISTENSEN and E. C. STAKMAN.

Barley plants were inoculated with 200 isolates from 8 genera of fungi; many of them caused leaf and sheath lesions as well as discolored kernels. Definite symptoms cannot be assigned to individual organisms, as there is great variation in symptoms, depending on environmental factors, varieties affected, the stage of development of the host, and the association of various organisms. Secondary invasions, especially by *Alternaria* spp., are common. Several bacterial isolates also were pathogenic. Isolations were made from about 500 samples of barley seed, mostly from Minnesota, and fungi belonging to more than 20 genera were isolated. In 1932 *Fusarium* spp. and *Helminthosporium* spp. were responsible for about 50 per cent of the shriveling and discoloration of kernels and, in 1933, for approximately 30 per cent. Evidently many other organisms

are important in causing defects on barley seeds. In both years Helminthosporium was more prevalent than Fusarium. Treating diseased seed lots with Ceresan resulted in improved stands and often in increased yields. Germination of seed on blotters is not a safe indication of performance in soil. (Minnesota Agricultural Experiment Station.)

Progress in the Control of Tobacco Downy Mildew. E. E. CLAYTON and J. G. GAINES.

Attempts to escape infection by locating beds on virgin land in remote places, sterilizing the soil, or treating the seed, have been ineffective. Tests of a large number of varieties of *Nicotiana tabacum* have shown all to be susceptible, with a Turkish type (Xanthia) least affected. *N. rustica* proved resistant. Recovered plants were distinctly more resistant to the disease than were plants not previously attached. Extensive spraying experiments have included consideration of schedules, methods of application, and materials. Among materials tested were the following sprays: Bordeaux, copper-soap, ammoniacal copper carbonate, copper acetate, oxide, sulphate, silicate, phosphate, and resinate, colloidal sulphur, potassium sulphide, calcium monosulphide, phenyl mercury acetate, oxyquinoline sulphate and cottonseed oil. Dusts used were: Bordeaux, copper-lime, copper carbonate, copper stearate, sulphur, calomel, and ethyl mercury phosphate. None proved appreciably better than the Bordeaux spray. In repeated tests with very young seedlings good disease control was secured, but extensive plant bed experiments in 1932 and 1933 at locations in 5 States gave mostly negative results. The disease usually developed after the plants had attained some size and adequate coverage was practically impossible. Spraying the larger plants may cause loss by reducing their ability to stand transplanting. Control of the disease was secured in plant beds heated at night to maintain 70° F. or above during the critical period. This control was obtained without modifying humidity relations or day temperature. It is believed that this method of protection would be less effective with very young plants.

Winter Injury and Drought in Relation to Apple Root Rot (Xylaria mali). J. S. COOLEY.

Winter injury is not uncommon in hillside apple orchards in Virginia and other eastern States. Some of this injury, which is usually on the larger roots or about the collar of the tree, occurred in the winter of 1932–33 and some in former years. It is much more severe on trees growing on the lower slope of the hill than on those near the crest. It is probable that poor aeration predisposes to injury by even moderate cold. The excessive dryness of the subsoil since the drought may have so changed the physical properties of the subsoil as to alter its seepage conditions. Black root rot (*Xylaria mali*) is much more prevalent on the lower slope of certain of these hillside orchards than on the higher and better-drained land, where winter injury is less pronounced. An examination of the trees affected with root rot indicates that winter injury may be a very important predisposing factor to infection by Xylaria root rot. Drought, as a contributing factor to the incidence of root rot, is attested by greater prevalence of disease on those trees located where they suffered from drought. The question of disease distribution, however, needs to be considered in a study of predisposing conditions.

Fungicidal Control of Gymnosporangium juniperi-virginianae and Related Species. IVAN H. CROWELL.

For the past 3 years experiments at the Arnold Arboretum of Harvard University have been carried out on fungicidal control of various species of Gymnosporangium, both with respect to red cedars and with pomaceous suscepts. Of the many fungicides tested, one has given satisfactory results—a special form of pure colloidal sulphur sup-

plied through the courtesy of a Boston chemical firm. This material has given essentially complete protection to pomaceous plants, as determined from hundred of controlled experiments carried out on a small scale. Direct attempts, with some interesting results in hand, to control various species of Gymnosporangium on red cedars are in progress. Of special importance is the possibility of controlling cedar-apple rust without having recourse to eradication of the red cedar.

Relative Susceptibility of the Species of Malus to Gymnosporangium juniperi-virginianae. IVAN H. CROWELL.

Opportunity has been afforded in the Arnold Arboretum of Harvard University for testing the susceptibility of all but 2 or 3 of the known species of Malus. The results show a range from high susceptibility to complete immunity. All species of the section Chloromeles of the genus Malus are susceptible. These species are native to eastern North America. Besides these, *M. fusca* of western North America is susceptible, though more resistant than the species of the section Chloromeles; the same is true of *M. sylvestris* of Europe. All other species (native to Eurasia) are immune. Complete series of tests were made with inoculum from Alabama, Kentucky, Massachusetts, Michigan, Mississippi, Nebraska, New York, and West Virginia. Interesting differences were noted in the aggressiveness of the pathogen from these areas—an indication of biological strains. It was also observed that the more highly susceptible species of Malus are liable to infection over a longer period than the less susceptible species.

Twig Blight (Hypomyces ipomoeae) of the American Bladder Nut. W. H. DAVIS.

Tips of dead twigs on bladder nut, *Staphylea trifolia,* were spotted with rose-colored spore masses bearing microconidia and macroconidia that were fusarial in appearance. This fungus is *Hypomyces ipomoeae,* for which bladder nut seems to be a new host.

The blight first appeared on young twigs about July 15th, and in wet, warm weather was accompanied by sporulation. From 10 to 50 per cent of the root sprouts may be killed and the main stem may be girdled and killed.

Infection was obtained by employing inoculum composed of viable conidia and ascospores. Wounding was not necessary for penetration and infection, but it aided. When leaves, old stems, maturing twigs, buds, and meristematic tissues were inoculated, only buds and meristem showed infection. Both conidial stages and the ascogenous stage formed in the host. Perithecia formed in the dead bark during late October and contained viable ascospores, which germinated best during the following May. The perfect stage was cultured in green, disinfected stems of the host, which were inoculated and incubated under laboratory conditions.

Hyphae remained viable in the pith and nodes of stems during the winters beginning with 1931 and with 1932.

Cortical Parasitism of Conifer-Seedling Roots in Pure Culture by Mycorrhizal and Nonmycorrhizal Fungi. K. D. DOAK.

A fungus, resembling *Rhizoctonia silvestris,* isolated from roots of *Pinus taeda,* not only formed the mantle and Hartig network characteristic of ectotrophic mycorrhizae on *P. taeda, P. resinosa, P. strobus, P. rigida,* and *P. radiata,* but also infected the cortex of mother roots. Although the latter type of infection was extensive, roots of *P. taeda* were not killed after 18 months in culture.

Two physiologic strains of *Armillaria mellea* grown with *Pinus strobus* and *P. rigida* infected the cortices of short roots and mother roots. Rhizomorph-like groups

of hyphae, penetrating the cortex, did not branch into individual cells or incite conditions indicating injury other than resin accumulation in cells near points of entrance. Seedlings of *P. strobus,* grown with one strain for 18 months, were not killed but showed more yellowing of fascicled needles than did the controls. Cork production at the points of emergence of lateral roots occurred in control cultures as well as with both strains of the fungus. One case of casual infection of a cork mass was found.

Fungi that Produce Ectotrophic Mycorrhizae of Conifers. K. D. DOAK.

In pure-culture syntheses the following combinations of fungi and coniferous seedlings gave typical ectotrophic mycorrhizae: *Boletus bicolor* with *Pinus rigida; B. granulatus* with *P. strobus* and *P. taeda; B. eximus* with *P. taeda; B. brevipes* with *P. rigida* and *P. taeda; B. chromapes* with *P. taeda; Boletinus pictis* with *P. strobus, P. taeda, P. resinosa* and *P. rigida; Cantharellus cibarius* with *P. taeda* and *P. strobus; Amanita muscaria* with *P. taeda* and *P. strobus; Russula lepida* with *P. rigida, P. taeda* and *P. strobus;* and *Scleroderma vulgare* with *P. strobus.* The mantle and Hartig network characterizing this form was demonstrated histologically in each case. The fungi were obtained in culture by isolations from the unexposed tissues of sporophores. Their growth was facilitated in the sand culture with sterile seedlings by use of 0.5 per cent dextrose in a mineral nutrient solution, and reisolations of the fungi from the synthesis cultures were successful in most cases.

Pythium butleri and P. aphanidermatum. CHARLES DRECHSLER.

Of nearly 100 cultures of Pythium isolated from various plants wherein the sporangia are often characteristically lobulate and the antheridium regularly consists of a swollen protuberant part broadly applied to the oogonium together with an unmodified portion of the supporting branch, 2 originating from diseased tobacco seedlings in Sumatra and 3 from affected sugar cane roots in Louisiana, apparently represent a different species from the others. The generally smaller zoosporangia here are derived from elements much less extensive, less swollen and less elaborately branched. The zoospores are smaller, their diameter, after encystment, averaging about 9μ instead of 11μ; and their production is difficult to induce on artificial substrata. The sexual structures are smaller, the oogonial diameter averaging about 22μ instead of 27μ, the oospore diameter about 17.5μ instead of about 22.5μ. The smaller antheridium is much more frequently produced immediately adjacent to an oogonium, though here also an adjacent antheridium generally fertilizes some less directly connected oogonium. Of the 2 species thus differing not only in dimensions (which, besides, vary considerably among the several strains) but also in reproductive behavior, the smaller one appears appropriately designable as *P. aphanidermatum,* the larger one as *P. butleri.*

Vascular Wilt and Root Rot of Pansies due to Aphanomyces sp. CHARLES DRECHSLER.

Late in April, 1933, at Mt. Rainier, Maryland, pansies in beds previously planted with pansies several years in succession, developed symptoms of wilt, which during the following weeks became increasingly severe. At this stage neither the stems nor the well-developed root systems of affected plants showed any evidence of cortical decay or of other external injury, but the vascular cylinder throughout was discolored deep red or orange red, very much as in some vascular wilt infections due to *Fusarium* spp. By the middle of May a general decay involved, in many plants, the base of the stems and the roots some distance downward, the aerial parts then collapsing, dying and drying up completely. In the earlier stages wilted plants revealed under microscopic examination numerous oospores immediately surrounding the discolored vascular cylinders

of infected roots. These oospores belonged to a species of Aphanomyces very readily and consistently obtained in pure culture from affected material. This species differs morphologically from *A. euteiches* from peas and does not show quite satisfactory agreement with the fungus obtained from the Centraalbureau voor Schimmelcultures at Baarn as *A. euteiches* P. F. 2.

Spraying and Dusting of Tomatoes for Late Blight (Phytophthora infestans). A. A. DUNLAP.

Copper-lime dust and 4–4–50 Bordeaux spray were found effective in controlling the late blight of tomatoes during the past season. Spray treatment gave larger yields of both ripe and green fruits than did the dust. Decay of the tomato fruits was almost completely controlled by spraying.

Seed Treatment Studies of Spinach. L. E. ERWIN and F. K. CRANDALL.

In greenhouse trials with 10 different chemicals used as seed treatments on various vegetable seeds it was found that the best control with spinach was obtained by the use of $CuSO_4$ (sol.), red oxide of copper, and P.D.-7. Further trials showed that P.D.-7 gave better control in every case, both in combating the preëmergence phase and the postemergence phase of the disease. P.D.-7 compared favorably with red oxide of copper as to adhesiveness. Trials in the field on spring and fall spinach with P.D.-7 and $CuSO_4$ (sol.) showed that the dust was just as effective as the $CuSO_4$ solution. The P.D.-7 gave a uniform stand and the seedlings appeared above the ground 2 or 3 days earlier than in the case of the $CuSO_4$ solution. Further trials with late fall spinach showed that P.D.-7 and red oxide of copper gave a vigorous and uniform stand, while the $CuSO_4$ solution showed a very uneven stand, and the damping off organism killed many of the plants after they appeared aboveground.

Comparing Soil Fungicide with Special Reference to Phymatotrichum omnivorum Root Rot. WALTER N. EZEKIEL and J. J. TAUBENHAUS.

Laboratory tests have been devised for comparison of the fungicidal value of materials after they have penetrated the soil. In closed jars of soil, surface applications of pentachlorethane, tetrachlorethane, xylene, carbon disulfide, perchlorethylene, trichlorethylene, dichlorethylene, turpentine, and paradichlorobenzene at 100 ppm. of soil weight penetrated 135 mm. of moist soil and prevented Phymatotrichum growth. Chloroform, dichlormethane, benzene, kerosene, ethyl chloride, ether, and naphthalene were less effective; and New Improved Ceresan, DuBay 1153A, DuBay 971A, Semesan, formaldehyde, and ammonia, ineffective even at 2000 ppm.

Direct toxicity tests of materials, after passage through air and cotton plugs, only, proved pentachlorethane, tetrachlorethane, xylene, and ammonia most toxic to Phymatotrichum. Carbon disulfide, chloroform, perchlorethylene, trichlorethylene, dichlorethylene, ethylene dichloride, and carbon tetrachloride were somewhat less toxic. Dichlormethane, ether, New Improved Ceresan, DuBay 1153A, paradichlorobenzene, formaldehyde, acetone, and benzene were still less toxic; and naphthalene, ethyl chloride, hexachlorethane, and alpha naphthol not toxic within the limits of the tests.

In preliminary field tests, tetrachlorethane injected 6 inches into the soil had, within 15 days, killed Phymatotrichum on infected cotton roots to at least 24 inches deep. Field tests with chlorinated hydrocarbons and xylene are being continued to determine their practical value in eradication of root rot.

Soil Treatment with Mercurials for Control of Potato Scab. C. W. FRUTCHEY and J. H. MUNCIE.

In 3 years' trials in the field and greenhouse, mercuric oxide, mercurous chloride and organic mercury, DuBay 965H, applied in the row before planting, failed to control soil-borne potato scab. Soils in these fields gave pH readings of 6.8–7.2. Materials were applied at 10, 20, and 10 pounds per acre, respectively. Of the 3 materials DuBay 965H showed a slight decrease of scabby tubers in only 1 test, while mercuric oxide and mercurous chloride increased the percentage of scabby tubers as well as area of tuber infection in every case. Applications of mercuric oxide and mercurous chloride decreased the ratio of soil bacteria and fungi to Actinomyces under controlled low-moisture conditions. Under high moisture conditions the ratio was increased. Several strains of *Actinomyces scabies* grew well in saturated solutions of mercuric oxide and mercurous chloride in tyrosinate liquid media. No correlation was found between tolerance to mercurials and virulence of *A. scabies.*

A Leaf Nematode Disease of Begonia. D. L. GILL.

The leaf nematode disease of begonia, caused by *Aphelenchoides fragariae,* is characterized by brownish discolorations on the under sides of the leaves. The disease is serious on the semituberous types of begonia grown under glass. Fibrous-root begonias are less susceptible. Migration of the nematodes occurs only when the plants are wet. The nematodes enter and emerge from the leaves through the stomata, are capable of living at least 81 days in detached leaves, and can exist in infested soil at least 6 weeks. If healthy and diseased leaves are maintained in direct contact the healthy individuals remain free from infection as long as the foliage is kept dry. Rapid spread takes place under the same conditions when the leaves are sprinkled. The thermal death point of the nematodes in the leaf tissue (47° C. for 5 minutes) is harmful to the plants. Fifty-six chemicals were tested for control without success. The disease, however, can be controlled by using healthy leaves for cuttings, avoiding infested soil, isolating diseased plants, removing infected leaves, spacing to avoid direct contact of plants, and keeping the foliage dry.

Sulphur Vaporation with Rupprecht's Sulfurator in Greenhouse-Crop Prophylaxis. E. F. GUBA.

This simple but remarkable invention for generating sulphur vapors from a specially constructed charcoal furnace promises to play a great rôle in crop-disease and insect control and to displace the use of other forms of sulphur fungicides and insecticides in greenhouse use.

In the past many commercial types of apparatus for vaporizing sulphur, notably Campbell's Sulfur Vaporizer, Rupprecht's Rota-Generator, Monro's Sulfur Generator, the Sulphur Fumigator of Cooperative Tuinbow Audoopvereniging, and simple home-made units comprising stove and container, have been used, but none approaches Rupprecht's Sulfurator in safety and practical use.

Control of the Narcissus Leaf-Scorch under Long Island Conditions. F. A. HAASIS.

Narcissus leaf-scorch (*Stagonospora curtisii*), although not a major disease in narcissus-bulb production, causes considerable reduction in crop yields. With certain exceptions, the varieties belonging to the *Poeticus, Leedsii,* and *Barrii* groups are more severely affected. Moderate temperature, precipitation, and an abundance of primary infections giving rise to secondary inoculum are important factors in promoting

epiphytotics. Soaking the bulbs in water for 1 hour, followed by a 30-minute dip in mercuric chloride 1–750 or formaldehyde 1–120 prior to planting, will greatly reduce primary infections. Secondary infections can be controlled with timely applications of copper fungicides, 4–4–50 Bordeaux spray or 20–80 copper-lime dust. The foliage should be protected from the first week in May until the third week in June. In general, this can be accomplished with from 4 to 5 applications. It is important, however, that the applications precede periods of moderate to heavy precipitation.

Toxic Action of Trichoderma on Rhizoctonia and other Soil Fungi. C. M. HAENSELER and M. C. ALLEN.

Trichoderma added to a soil heavily infested with Rhizoctonia reduced seed decay and damping off on cucumbers and peas from 67 and 63 per cent to 12 and 38 per cent, respectively. With a Pythium-infested soil the reductions were from 69 and 77 per cent to 12 and 47 per cent, respectively. A liquid nutrient medium in which Trichoderma had grown for 5 days, sterilized by passing through a Seizts filter, proved to be lethal to Rhizoctonia and Pythium. It was necessary to dilute this toxic filtrate to approximately ½ its concentration with water, fresh nutrient, or "deactivated" filtrate before growth could take place. The toxic properties of the filtrate were destroyed by heating for 10 minutes at 80° C., by aging in cotton plugged test tubes or flasks for 10 days, or by bubbling oxygen through the medium for 5 minutes. Preliminary attempts to concentrate the toxic principle by precipitation with alcohol were unsuccessful.

The results indicate that the occurrence of Trichoderma in the soil may have an antagonistic effect upon Rhizoctonia and possibly other soil-borne plant pathogens, and that this antagonism may in part be due to toxic materials elaborated by the Trichoderma.

Preliminary Note on the Relation of Mycorrhizae to Dry-Weight Increase in Pinus strobus. A. B. HATCH.

Dry weights of 3-month seedlings grown from weighed seeds in pot cultures (27 seedlings each) using soils of different fertilities, were examined for simple correlation with numbers of mycorrhizae. Seedlings in 5 out of 37 pots yielded significant values for r (Fisher's table of t)—3 positive and 2 negative. In one case duplicate pots gave significant correlations of opposite sign, revealing the fortuitous character of significant values of r for this material. Accordingly, the relationship was apparently casual. It seems permissible, therefore, to predict that mycorrhizae were not detrimental.

A similar prediction concerning their possible beneficial rôle is not justified, since masking of beneficial effects could occur, in that: (1) low internal nutrient concentrations are seemingly responsible for mycotrophism in Pinus. Therefore, seedlings that initially develop mycorrhizae possess low, internal nutrient concentrations, and those that remain nonmycorrhizal possess high ones. (2) The beneficial theory postulates superior absorptive efficiency for mycorrhizae, hence seedlings differing in nutrient reserves at germination should tend toward uniformity when exposed to mycorrhizal inocula. Apparently, this may occur, since seedlings grown in soil cultures have lower coefficients of variation than sand-culture seedlings, where mycorrhizal inocula are lacking.

Effect of Air Temperature on Tobacco Ring-Spot Infection. R. G. HENDERSON.

Studies conducted in the greenhouse and in incubators indicate that the development of ring-spot symptoms, following artificial inoculation, is influenced by the air temperature in which the plants are grown. At temperatures above 93° F. symptoms did

not develop, but at 80–85° symptoms developed in 4–5 days. Plants with primary lesions on the inoculated leaves, when transferred from a low temperature (80–85°) to a high temperature (above 93°), did not develop further symptoms. On the other hand, plants, held at a high temperature for several days and on which no symptoms had appeared, developed primary lesions on being transferred to a low temperature.

Experiments on the Control of Downy Mildew of Tobacco. R. G. HENDERSON.

Several fungicides were used on plant beds under field conditions last spring in an attempt to find one that might be effectively used in controlling tobacco downy mildew (*Peronospora hyoscyami*). Of the fungicides used in the experiment, calcium sulphide (Cal-Mo-Sul) alone gave results approaching commercial control. Bordeaux gave only slight control. Similar results have been secured by experiments conducted under greenhouse conditions. It seems necessary, however, to make more frequent applications in the greenhouse than in the field in order to get satisfactory control. Although the available data are not conclusive, calcium sulphide apparently is a promising fungicide for downy-mildew control in tobacco-plant beds. Where frequent applications of calcium sulphide have been made on small seedlings in the greenhouse, there has been a noticeable stunting of plant growth.

The Origin of Roots Stimulated by Hairy-Root Bacteria in Apple Stems. E. M. HILDEBRAND.

The disease of nursery apple trees known as "infectious hairy root" is of interest to botanists generally because the causal organism, *Phytomonas rhizogenes*, stimulates the formation of roots. Hairy roots originate in the first stages of the disease over a period of approximately 4 weeks after inoculation. They begin in the callus-disease tissue, elaborated by the host, due to the combined effects of wounding and the presence of the bacteria. Wounding alone apparently stimulates the ray cells of the xylem and cells of all the living tissues outside the woody cylinder, except the epidermis (periderm), to multiplication, which may soon cease with the reestablishment of cambial continuity. The presence of the bacteria, however, largely prevents this reestablishment and there is further proliferation and root formation. The first and second weeks are marked by cell multiplication over practically the entire face of the wound. When present, bacteria, crushed cells, and foreign matter may become entirely surrounded. The resulting islands appear as somewhat circular areas of hyperplasia. Beginning late in the second week meristematic localities appear in the elaborated tissues from which the roots later develop quite independently of the deeper normal tissues.

Masked Strain of Tobacco-Mosaic Virus. F. O. HOLMES.

A study has been made of a strain of tobacco-mosaic virus, the effects of which are masked in *Nicotiana tabacum*. The masked strain and the ordinary strain of tobacco-mosaic virus are alike in their responses to heating, storage, and dilution and in their host range. In all necrotic-type hosts primary lesions of the strains are indistinguishable in appearance. In hosts in which systemic necrosis or intense yellowing are produced by the ordinary strain the symptoms of the masked strain are less severe. Hosts in which mottling is produced by the ordinary strain act as symptomless carriers of the masked strain. In tobacco both the masked and the ordinary strain of tobacco-mosaic virus cause abnormal starch retention in recently affected leaf tissues. This effect can be made conspicuous by removal of chlorophyll, and subsequent staining with

iodine. The areas of young leaves thus shown to be affected differ for the 2 strains, systemic spread of virus being less extensive in plants infected with the masked strain.

Zinc Oxide as a Seed and Soil Treatment for Damping Off. JAMES G. HORSFALL.

The red copper oxide seed treatment recently proposed (N. Y. S. Agr. Exp. Sta., Bul. 615) for damping off increases stands markedly and widely, particularly in field crops. Sometimes, however, it is not wholly satisfactory for prevent post-emergence damping off in greenhouses caused by *Pythium ultimum*. A search was instituted to discover a chemical having persistent fungicidal properties applicable to the soil surface without producing undue plant injury. Soluble zinc compounds, having been used as wood preservatives and for fire-blight control, were tried and found to be fungicidal but excessively toxic to the plants. Moreover, their fungicidal effect was transitory.

Zinc oxide, under test since January, 1931, is insoluble in water, therefore persistent, is relatively noninjurious when applied before emergence, and is satisfactorily fungicidal. Being insoluble, it penetrates poorly into soil; hence is inefficient for increasing stands, a difficulty easily overcome by mixing it with the covering soil or by using it with a seed treatment. Zinc oxide itself is an effective seed treatment, sometimes appearing superior to red copper oxide. Used at 10 to 20 grams per square foot, it has been commercially successful in greenhouses in several New York State localities. It has been applied to transplanted seedlings but occasionally injury results.

Correlation Between Rough-Hairy Pubescence in Soybeans and Freedom from Injury by Empoasca fabae. E. A. HOLLOWELL and H. W. JOHNSON.

To determine if the field-observed freedom of rough-hairy varieties of soybeans from the injury caused by the potato leaf hopper is correlated with the occurrence of rough-hairy pubescence in material of known genetic constitution, the writers obtained from C. M. Woodworth of the Illinois Agricultural Experiment Station seed of 29 glabrous F_2 plants from a cross between Illini (rough-hairy) and a dominant glabrous type of soybean. Of the F_3 progenies from these plants 14 were homozygous-glabrous and 15 were heterozygous, segregating in a ratio of approximately 3 glabrous to 1 rough-hairy. Of the F_4 progenies, 47 were homozygous-glabrous, 32 heterozygous, and 29 homozygous rough-hairy. Of the F_5 progenies, 32 were homozygous-glabrous, 32 heterozygous, and 9 homozygous rough-hairy. In the 3 generations grown the glabrous individuals (total number 8570) of both the homozygous-glabrous and heterozygous progenies were all heavily infested with Empoasca, severely stunted in growth, and had curled leaves with yellowed, necrotic margins. The rough-hairy individuals (total number 6755), on the other hand, were almost entirely free from Empoasca, grew vigorously, and their leaves showed no symptoms of leaf-hopper injury. It is concluded that in soybeans, at least with the material under test, freedom from injury by the potato leaf hopper is correlated with the occurrence of rough-hairy pubescence.

Partial Recovery and Immunity of Virus-Diseased Abutilon. JOHN Y. KEUR.

It has been noted frequently that variegated Abutilon plants may recover entirely or partially from the effects of the virus. In case of partial recovery, green-leaved branches develop on variegated virus-diseased plants. Baur claimed that such branches, developing on *Abutilon thompsonii*, were immune. The following results were obtained with green branches of 5 variegated Abutilon species: (1) The virus is absent in such branches as demonstrated by the inability of scions to infect susceptible green Abutilon clons. (2) The virus does not pass through such green branches in sufficient quantity to produce variegated symptoms in susceptible green scions grafted on the green

branches. (3) These green branches are not actually immune. Green clons propagated from them can be reinfected not only by scions of other variegated Abutilon clons, but also by scions of their own variegated clons.

Tobacco and Aucuba-Mosaic Infections by Single Units of Virus. L. O. KUNKEL.

When two samples of tobacco juice containing the viruses of aucuba and tobacco mosaics, respectively, are mixed in equal or other proportions up to one part of either in 30 parts of a mixture and used to inoculate mature leaves of *Nicotiana langsdorffii* plants by the needle-puncture method, circular necrotic lesions of uniform size and color develop around those punctures at which infection occurs. By subinoculations from these leaves to plants of *Nicotiana sylvestris* it is possible to show whether any given lesion contains tobacco mosaic virus, aucuba mosaic virus, or a mixture of the two. By making use of this technique in the study of a large number of infections, it has been shown that needle-puncture inoculations from mixtures of the 2 viruses give lesions containing either one or the other, but seldom both. Since each virus is readily transmitted by the needle-puncture method, the data obtained bring evidence that, except in a small percentage of cases, infection results from a single virus unit. The method is, therefore, suitable for the isolation of pure cultures derived from single units of either virus. The lesions are comparable to bacterial colonies isolated by the Koch plate method.

Experimental Production of Crown Gall on Opuntia. MICHAEL LEVINE.

Attempts of the writer to induce the crown gall disease on Opuntia species have up to the present been unsuccessful. *Opuntia Keyensis* after seven years produced large, well developed galls after inoculation with *B. tumefaciens.* The growth appeared to form four months after the inoculation of young well-rooted joints. One year after inoculation the galls attained the size of a black walnut, and at present show no tendency to ageing or necrosis. Microscopical studies of this gall show a disoriented conglomeration of cells consisting of fibro-vascular elements, clusters of embryonic cells and parenchymatous tissue surrounded by fragments of epidermis and bark. There are also a number of definitely organized structures which to all appearances are embryonic roots developing in the periphery of the crown gall. Many host cells, as well as crown-gall cells, contain aggregates of crystals while only the host tissue contains mucilage or slime cells. The majority of Opuntias so far studied appear to lack the protecting mechanism of forming overgrowths common to a large number of dicotyledonous plants in that the introduction of the tumor-producing organism results in injury and death of the tissue. Laboratory Division, Montefiore Hospital, N. Y. C.

The Production of an Apparently New Variety of Puccinia graminis by Hybridization on Barberry. M. N. LEVINE, RALPH U. COTTER and E. C. STAKMAN.

In an effort to determine the extent to which new parasitic strains of *Puccinia graminis* may result from hybridization between existing strains on barberries in nature, artificial crosses were made in the greenhouse between certain physiologic forms of *P. graminis tritici* and *P. graminis secalis.* As a result there were obtained lines that attack certain varieties of barley normally but not the other cereals. As the new lines seem restricted to barley, they could properly be designated as a variety. The following crosses were made: *P. graminis secalis* 7 × *P. graminis tritici* 21 and *P. graminis secalis* 7 × *P. graminis tritici* 101. In both cases the progeny were similar. There is some evidence also that *P. graminis tritici* 34 × *P. graminis agrostidis* produced a rust

similar to the above.—(Division of Barberry Eradication and Division of Cereal Crops and Diseases, U. S. Department of Agriculture, in cooperation with the Minnesota Agricultural Experiment Station.)

Some Aspects of the Pathology of Forest Trees and Forest Products in Middle Europe and Scandinavia in 1933. R. M. LINDGREN.

Nutritional Relationship in the Apple-Rust Fungus. J. C. LIU.

In apple rust the aecium began its formation about 40 days after inoculation, although the epidermis was not ruptured until 20 days later. Toward the end of the first month after inoculation the spermogonium is in full development but the mycelium can hardly be distinguished under the microscope. In 30–40-day-old lesions, however, the mycelium appears quite vigorous. After the formation of the aecia the mycelium is again indistinct. Such behavior of the mycelium is explained as follows: The spermogonium, being a large organ, with multiple functions to perform, naturally requires an abundance of food, which can be supplied only by the mycelium. Due to this drain, the mycelium becomes depleted. After the spermogonium has completed its development and activities, the demand for food decreases. Consequently, the mycelium becomes replenished and is in a condition to initiate the aecium. Development of the aecium means a further food requirement. The food supply is diverted from the spermogonium to the newly-formed aecium. Consequently, the former dies of starvation and its exudation ceases.

Inheritance of Resistance to Powdery Mildew, Erysiphe graminis tritici, in Wheat. E. B. MAINS.

The inheritance of the resistance of Norka C. I. 4377 to physiologic form 1 of *Erysiphe graminis tritici* has been studied in crosses between Norka and the susceptible varieties Webster, Ceres, Chinese, Kota, Malakoff, Reliance, and Warden. In these crosses resistance was inherited as a simple dominant factor, the segregation in the F_2 being approximately 3 resistant : 1 susceptible. Resistance to mildew was inherited independently of the resistance of Norka to physiologic form 3 of leaf rust, *Puccinia rubigo-vera tritici.* The resistance of Red Fern to mildew has been found to be inherited also as a simple dominant Mendelian factor. The resistance of Hope C. I. 8178, in crosses with the susceptible varieties Chinese, Marquis, Michigan Amber, and Reliance, was inherited as a simple, recessive Mendelian factor, the ratio in the F_2 being approximately 1 resistant : 3 susceptible. The resistance of Sonora and the resistance of Michigan Amber 29–1–1–1 in crosses with Chinese were each also inherited as a simple recessive factor.

Relative Infectivity of Mosaic Virus Extracted from Various Parts of Sugarcane. JULIUS MATZ.

Using methods of inoculation found superior to previous methods for insuring infection with extracted juice (Jour. Agr. Res. 46 (9): 821, 1933) tests were made to find whether, in an infected plant, there might exist specific regions in which the activity of the virus is, at least partly, normally inhibited or inactivated. Results to date show that in individual shoots juices from infected expanded lamellae of young and mature green leaves, midribs, leaf sheaths, and true stems, separately, are equally infectious to a high degree (80–100 per cent), whereas the juice from the rolled inner white and brittle portion of the leaf bases in the lower region of the pseudostem, from the same individual shoots in parallel series of inoculations made at the same time

was comparatively low in infectivity (0–50 per cent). The symptoms and their time of appearance in all tests showed no deviation in any noticeable manner from normal virulent sugarcane mosaic, indicating no qualitative change but suggesting a quantitative reduction of virus in the juice expressed from the inner white false stem as compared with the juice from the other sources. However, the addition of equal volumes of healthy false-stem juice to virulent green-foliage juice after extraction did not result in low infectivity, nor was there any reduction in infectivity when infectious green-foliage juice was diluted with an equal volume of healthy green-foliage juice.

Susceptibility of Treated and Untreated Turf to Brownpatch and Dollarspot. H. F. A. NORTH and L. E. ERWIN.

During the progress of experiments carried on by the Rhode Island Experiment Station, many of the plats of fine turf have been heavily damaged by brown-patch (*Rhizoctonia solani*) or by dollarspot (*Rhizoctonia* spp.). The average percentage of area affected by these diseases in 1931 to 1933 has been computed for 15 plats of colonial bent, *Agrostis tenuis*, 19 plats of velvet bent, *Agrostis canina*, and 19 plats of creeping bent, *Agrostis palustris*. Marked seasonal and varietal variations in disease have been the rule, but in general, grasses have tended to continue susceptible to one or both diseases from year to year. Colonial bent plats have been susceptible to brown-patch but highly resistant to dollarspot. Most of the strains of velvet bent appear rather resistant to brownpatch but susceptible to dollarspot. Creeping bents, though more variable than the two other species have resembled velvet bent in susceptibility. Good control of these diseases has been obtained by the use of bichloride of mercury 1/3, and calomel 2/3, but much depends upon local weather conditions. A test of trade products advertised for the control of brownpatch shows that few are more effective than the mixture cited above and a number that are much less useful.

Incubation Period of Pea Mosaic in Macrosiphum pisi. H. T. OSBORN.

The pea aphis has been used as a vector in the transmission of pea mosaic to the broad bean, garden pea, sweet pea, and Canada field pea. Because of the ease with which it may be grown, the broad bean was used in most experiments. It has been found that the pea aphis, after feeding on infected plants, acquires the ability to infect healthy plants and that this ability is retained throughout the active feeding life of the insect. By exposing a succession of healthy plants to colonies that were allowed to feed for a short period on diseased plants, an incubation or noninfective period has been shown to exist. In these experiments the period varied from 12 to 28 hours. Mechanical transmission of pea mosaic to the broad bean is difficult.

Rosette of Blackberries and Dewberries. A. G. PLAKIDAS.

The rosette, which is apparently identical with the "double blossom" disease of the Southeastern States, is the most serious disease of blackberries and dewberries in Louisiana. Although the double blossom disease has been generally attributed to *Fusisporium rubi*, studies made in Louisiana indicate that the causal agent of the rosette is a species of Cercosporella. The disease was repeatedly produced by inoculation with pure cultures of this fungus. Natural infection takes place on the primocanes in the spring, but the symptoms do not appear until the following spring when these canes resume growth. At that time the infected buds give rise to witches' brooms, and to abnormal blossoms that do not set fruit. The period during which infection takes place is from about the middle of March, when the spores begin to appear on the diseased blossoms, to about the middle of June. Spores are found in abundance on the withered blossoms until the

last part of August, but very little infection occurs after the middle of June. Spraying the primocanes with 4–4–50 Bordeaux every 10 days from the first of April to the first of June gave practically complete control (about 98%) of the disease.

Sweet-potato Ring Rot Caused by Pythium ultimum. R. F. POOLE.

A specific soft decay of sweet potato, ring-like in character, working from one side of the root to another without involving all tissues, is found to be due to *Pythium ultimum* and not to *Nectria ipomoeae* nor to *Rhizopus nigricans*, which were for some time attributed causes.

The fungus works intercellularly and intracellularly; produces amylase, pectinase, and protease but, apparently, no diastase. Rapid decay occurs in 36 to 48 hours and complete rings develop in 3 days at a temperature of 20° C. in an atmosphere saturated with moisture.

Ring rot developed after inoculation in all commercial varieties of sweet potatoes. The Jersey and Nancy Hall varieties were apparently most susceptible.

The disease was severe on sweet potatoes harvested after rains maintained high soil moisture conditions in late autumn. It was of no importance on sweet potatoes harvested prior to the rains.

Apple Target Canker, Measles, and Rough Bark. JOHN W. ROBERTS.

A bacterium, probably *Pseudomonas papulans*, which Rose described as the cause of apple blister-spot and probably the cause of apple measles in Missouri, has been isolated from the papulate early stage of apple target canker. Although inoculation of "Delicious" apple twigs has been followed by lesions typical of the early stages of the disease and the bacterium has been reisolated from these lesions, occurrence of the disease on noninoculated checks made the results inconclusive. Isolation of what appears to be the same organism from the papulate early stages of the rough-bark disease of the Yellow Newtown apple throws doubt on a former assertion of the writer that the rough-bark disease is caused by *Phomopsis mali*. Apple measles, target canker, and the rough-bark disease may prove to be identical, since their early stages are alike and what appears to be the same species of bacterium has been isolated from the early stages of all of them. They may also be identical with the apple diseases with which Lacy and Dowson in England and Hopkins in South Africa found organisms similar to, if not identical with, *Ps. papulans* to be associated.

Studies on Barley Smut in 1933. MABEL L. RUTTLE (MRS. NEBEL).

Further studies on the barley-smut types described by the writer in 1932 have been made. Seed inoculations with *Ustilago hordei* and Types 2, 3, and 4 and blossom inoculations with *U. nuda* have produced the respective types, with certain exceptions. The description of Type 6, only, has been altered. It is now regarded as having a loose head, and chlamydospores, olivaceous-brown in mass, which germinate by promycelia and sporidia rather than by germ tubes, as is more usual with spores of this color. Type 4 corresponds closely to the descriptions of *U. nigra* Tapke (1932) and *U. medians* Biedenkopf (1895).

Crosses between monosporidial lines of *Ustilago hordei* (covered head) and Type 3 (loose head) have been made. The F_1 smutted heads resembled those of Type 3, but are possibly slightly more covered. The chlamydospores are smooth, like those of the parents, and germinate readily by promycelia that cut off sporidia. The sporidia culture easily. Seed inoculated with bisporidial cultures of *U. hordei* and Type 3 gave plants infected with the respective parental types.

Tennessee Winter barley was resistant to the form of *Ustilago nuda* collected on Featherston barley and susceptible to *U. hordei* and Type 4. Featherston barley was not infected with one collection of Type 4 but was infected by *U. hordei*, *U. nuda*, and Types 2, 3, and another collection of Type 4. Alpha barley was susceptible to all collections of the different types so far studied.

Components of Potato Mild Mosaic. E. S. SCHULTZ, R. BONDE and W. P. RALEIGH.

Mild mosaic, manifested by irregular light green spots and by slight wrinkling on Green Mountain potatoes, has been found to consist of 2 components. These components are a latent one that is masked in the variety Green Mountain as well as in many other varieties, including a Green Mountain seedling; and a second component, manifested on a seedling potato resistant to the latent component and on a latent-free Green Mountain seedling by light green and slightly rugose leaves. The second component has been separated from the latent component by means of aphids, which transmit the second but not the latent component. It also has been separated by means of a seedling potato, which takes the second but is resistant to the latent component. Inoculating healthy Green Mountain seedlings with both of these components produced typical mild mosaic. Moreover, inoculating Green Mountains harboring the latent component with the second component yielded mild mosaic. The reaction of latent-free Green Mountains to the second component has not been determined.

Intercellular Relative Humidity in Relation to Fire-Blight Resistance in Apple and Pear. LUTHER SHAW.

Resistance to *Erwinia amylovora* (*Bacillus amylovorus*) in apple and pear tissues varies greatly as the environment, particularly the amount of moisture, is varied. Studies were made to determine if these variations in resistance are related to differences in the relative humidity in the intercellular spaces of the host tissues. Measurements were made of growth of the blight pathogen on a variety of culture media in equilibrium with the following relative humidities: 99.9 (approximate), 99, 98, 97, 96, and 95 per cent. These humidities are approximately equivalent to osmotic pressures of 2, 12, 27, 41, 55, and 68 atmospheres, respectively. Maximum growth was obtained at 99.9 per cent relative humidity; at 99 per cent growth was reduced to approximately ½ of maximum; at 98 per cent approximately 1/10; and at 97, 96, and 95 per cent almost no growth occurred. Measurements were made of the relative humidity in the intercellular spaces and the relative fire-blight resistance of apple and pear tissues at different soil moistures and atmospheric humidities. Results of these tests showed a direct relation between increased fire-blight resistance and lowered relative humidity in the intercellular spaces of the tissues. Ninety-seven per cent relative humidity in the intercellular spaces was associated with complete resistance to infection and 100 per cent with maximum susceptibility.

Persistence of Heart-Rotting Fungi in Girdled Trees. PERLEY SPAULDING.

Among various features that have marked recent developments in forest pathology is the fate of heart-rotting fungi when their host trees are killed by girdling. Examination of 1,768 girdled trees, principally red maple, sugar maple, yellow birch, beech, and paper birch that have been girdled from 2 to 5 years has shown 80 bearing living sporophores of 5 different species of heart-rotting fungi, which certainly were established in the interior of the trunks before the girdling. This is a large enough number to serve as infection sources for the young stand. Older girdled trees will have to be examined to learn how long these fungi may persist in the dead trunks. In another

5 years most of them will be uprooted and fallen over. This will quite certainly result in the dying out of some of the fungi. However, if the snags fall in such a way that they can still soak moisture from the ground in any quantity the fungi will continue to fruit. But a relatively small percentage of them is likely to do this. Girdling or felling of infected trees will not result in a complete killing of these fungi, if there is no further disposal of the down stuff. Both will greatly reduce the number of living fungi and probably will almost eliminate them before a second stand of young trees reaches a susceptible age.

The Pathogenicity and Cytology of Urocystis occulta. E. C. STAKMAN, M. B. MOORE and R. C. CASSELL.

Inoculated rye seed was planted in soil maintained at the temperatures given, until plants emerged. The following percentages of smutted plants resulted: at 14° C., 47 per cent; 19° C., 35 per cent; 24° C., 24 per cent; 29° C., 0 per cent. In cooperation with Minnesota agronomists, 139 selfed lines of rye were tested for resistance. They ranged from highly resistant to completely susceptible. Eight varieties of rye were inoculated with 5 collections of smut, but there was no conclusive evidence of physiologic specialization. Opportunity for variation resulting from hybridization in *Urocystis occulta* appears more restricted than in those smuts whose sporidia become abjointed from the promycelium and bud extensively. Chlamydospores of *U. occulta* produce a promycelium on which are formed 2 to 6 uninucleate sporidia that apparently do not bud nor become abjointed. Sporidia on the same or different promycelia fuse in various ways, giving rise to binucleate hyphae of the dikaryophase, although occasionally sporidia appear to become binucleate without previous fusion. Vegetative hyphae in the host appear predominately binucleate, the diplophase resulting from nuclear fusions in the spore-mother cells.

The Action of Trypsin on Tobacco-Mosaic Virus. W. M. STANLEY.

The addition of a solution of crystalline trypsin to the nontreated juice of mosaic-diseased tobacco plants (*Nicotiana tabacum* var. Turkish) immediately rendered the juice practically noninfectious. Addition of trypsin to highly infectious purified solutions of tobacco-mosaic virus gave similar results when tested on *Phaseolus vulgaris* var. Early Golden Cluster, *N. glutinosa*, and *N. tabacum* var. Turkish. In many instances the infectivity of the virus was reduced to zero within 1 minute after the addition of the trypsin, yet no digestion of protein was detected by chemical analysis. The infectivity of the virus-trypsin solutions increased on dilution, standing, or moderate heating. The addition of globin and trypsinogen, which have no proteolytic activity, but have isoelectric points near that of trypsin, gave the same result as did the addition of trypsin. Pepsin, gelatin, and egg albumin, all of which have isoelectric points towards the acid side, were noneffective. The results indicate that the reduction in infectivity of the tobacco-mosaic virus is not due to the proteolytic activity of trypsin.

Longevity of Sclerotia of Phymatotrichum omnivorum in Moist Soil in the Laboratory. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Phymatotrichum omnivorum is known to survive in vegetative condition on roots of susceptible plants, and in dormant condition as sclerotia or strand sclerotia in the soil. Control of the disease by rotation with resistant crops depends on knowledge of the longevity in the soil of both stages of the fungus.

In October, 1929, sclerotia recovered from an infested carrot field at Temple, Texas, were placed in a number of small corked vials of moist Houston clay soil material and

stored in the laboratory. Each year 50 sclerotia have been tested for germination on moist filter paper in Petri dishes. Initial germination tests showed 68 per cent of the sclerotia viable. One year later, germination had decreased to 46 per cent, and in successive years to 28 per cent, 21 per cent, and 11 per cent, respectively. Some of the sclerotia that were still viable and germinated after 4 years' storage were placed on roots of normal cotton plants growing in glass jars. These sclerotia produced Phymatotrichum strands that attacked the cotton roots and eventually killed the plants, causing symptoms typical of Phymatotrichum root rot.

Two New Diseases of the Texas Bluebell, Eustoma russellianum. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Florists growing the Texas bluebell, *Eustoma russellianum*, have, during the last 5 years, experienced serious difficulty from diseases attacking this plant. Study showed that 2 distinct diseases were involved. One was a crown rot and damping off, caused by *Fusarium solani*. The second was a stem and leaf blight, which was proved to be caused by another fungus, *Sclerophoma eustomonis*, n. sp. Pure cultures of both organisms were isolated from infected material, the respective diseases reproduced artificially by inoculation of healthy plants, and the two organisms recovered again. Viable spores of both organisms have been found on insects from diseased plants. The fusariosis was controlled by soil sterilization with formaldehyde. The stem blight was controlled by spraying with 4–4–50 Bordeaux mixture plus casein as a spreader and nicotine sulphate as an insecticide.

Further Studies on a Noninfectious Leaf-Deforming Principle from Mosaic Tomato Plants. M. H. THORNTON and H. R. KRAYBILL.

When the expressed juice of mosaic tomato plants was autolyzed and the virus inactivated by boiling, the juice was found to contain the noninfectious, leaf-deforming principle described by Kraybill and Eckerson and by Kraybill, Brewer, Samson, and Gardner. The activity of the juice was not diminished by precipitation of the proteins with alcohol or acetone nor by extraction with ether. Numerous attempts to obtain active preparations from healthy plants were unsuccessful. Preparations from the fresh juice of tomato plants showing "fern-leaf" symptoms produced by inoculation with cucumber mosaic were inactive. Tomato plants showing marked symptoms produced by inoculation with the noninfectious leaf-deforming principle and tomato plants showing typical symptoms of mosaic were lower in reducing sugars, sucrose, starch, and hemicellulose and higher in total nitrogen and nitrate nitrogen than healthy plants.

Susceptibility Reactions of Pinus sylvestris to Woodgate Rust. R. P. TRUE.

Eosin-conduction experiments were made upon 1-, 2-, and 3-season material of infected and noninfected portions of susceptible twigs on August 18. These indicated that, while at first all the gall wood conducts, in the second season there appears a nonconducting core, which is later filled with discoloring depositions. By the third season this nonconducting, discolored core may either approach the cambium closely or occupy a comparatively small area at the center of the gall. In one case of the former the distal portion of the branch was dwarfed and dying. Following anatomical studies, infected and noninfected areas of susceptible twigs of corresponding ages were collected on September 20 and tested for cellulose, lignin, pectic compounds, starch, fats and oils, tannins, and resins and terpenes. Infected areas showed comparatively more of the reserve food substances and tannins. The number of resin canals was increased, but not disproportionately, to the increased volume of gall wood. Lignification

was suppressed to some degree by the presence of the fungus in the cambium. This tendency was especially pronounced near cambium areas that were being actively invaded.

Purification of the Virus of Tobacco Mosaic. CARL G. VINSON.

Juice from diseased plants may be increased in infective power more consistently by the addition of activated charcoal following the addition of Lloyd's reagent. A small amount of normal solution of aluminum sulphate produces a flocculent precipitate when added to the virus fraction obtained on decomposing the safranin precipitate of the virus with Lloyd's reagent. This precipitate packs sufficiently to allow complete decantation of the supernatant liquid. Very little activity has thus far been demonstrated in the supernatant liquid. The precipitate contains the infectious agent.

Magnesium sulphate also may be used to salt out the virus from the above-mentioned fraction, but a much greater quantity is required.

The Stimulation of Fungus Spore Germination by Aqueous Plant Extracts. FRANK WILCOXON and S. E. A. MCCALLAN.

In the course of toxicity studies difficulty is experienced in germinating in distilled water the spores of many species of fungi. Conidia of *Penicillium* sp. and *Neurospora sitophila* will not germinate at all, while the conidia of *Pestalotia stellata, Glomerella cingulata, Botrytis paeoniae,* and *Sclerotinia americana,* because of age or other factors, often give unsatisfactory germination. The addition of small quantities of tomato, orange, apple, or pear juice and aqueous extract of lily or gladiolus bulbs and dahlia or potato tubers resulted in nearly 100 per cent germination. Yeast extract and urine also stimulated. Extracts from *Coprinus micaceus* and *C. atramentarius* and tomato leaves did not stimulate. The stimulation afforded by standard nutrient solutions is greatly inferior. About 0.05 per cent of solids from tomato juice or yeast extract in the spore suspension will give rise to almost 100 per cent germination in Penicillium and Neurospora. The stimulating factor is heat-stable and dialyzable. Ascorbic acid (vitamin C) did not stimulate, nor did inositol when added to Bios II according to the method of Lash Miller. This factor appears to resemble the "Rhizopin B" of Nielsen.

The Diurnal Cycle of Erysiphe polygoni. CECIL E. YARWOOD.

Under field and greenhouse conditions the powdery mildew of red clover has shown a daily periodicity with respect to the division of the generative cell of the conidiophore, the maturation of the conidium, the discharge of the conidium, the conidial germinability, and the ability of the conidia to cause infection. The generative cell divides during the day and the distal of the 2 daughter cells develops within 2 days into the mature conidium. Typically, each conidiophore matures a single conidium per day, which is ordinarily liberated about noon. Spores taken from mildewed plants in the late morning and afternoon show a high germinative capacity, whereas spores taken from plants late at night or early in the morning germinate poorly. Inoculations made during the day result in a higher percentage of infection than those made at night. The diurnal cycle is no longer evident under conditions of continuous artificial light and is less marked during cloudy weather. In alternate artificial light and darkness (12 hours each) with temperature and humidity fairly constant, the diurnal cycle is similar to that under usual field and greenhouse conditions. *Erysiphe polygoni* on *Delphinium* spp., in the greenhouse, showed a similar diurnal cycle with respect to conidial formation and germinability.

(Cooperative investigations between the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

Sclerotium Blight Destroys Winter Wheat in Gallatin County, Montana. PAUL A. YOUNG.

Many sclerotia were found in the field on the straw of Turkey wheat, *Triticum aestivum*, and on dead stems of *Sisymbrium altissimum* in April, 1932 and 1933. Evidently these were sclerotia of *Sclerotium fulvum*. They were found only on land where Sclerotium-killed wheat leaves were collected then and during the preceding year. Cultures from the sclerotia on the overwintered wheat and mustard stems slowly produced sparse, hyaline hyphae and many sclerotia on cornmeal agar at 5° C. These turgid sclerotia were reddish brown and 700 to 1200μ in diameter, with aggregate sclerotia 2200μ in diameter. When dry, these sclerotia were black and 200 to 900μ in diameter. Snow, melting from infested fields, revealed white, tan, or gray, unshriveled wheat leaves appressed to the soil. These leaves, killed by *Sclerotium fulvum*, contained many reddish brown to black sclerotia 200 to 500μ in diameter. They did not produce a perfect stage in many experiments. Wheat, surviving winter blight, bore light-colored heads with shriveled grain. Forty varieties of wheat showed no resistance to natural infection by *Sclerotium fulvum*. Fall-sown wheat, emerging in the spring, escaped Sclerotium blight.

VARIABILITY IN MONOCONIDIAL CULTURES OF VENTURIA INAEQUALIS[1]

D. H. PALMITER[2]

(Accepted for publication March 14, 1933)

Apple scab and its casual organism *Venturia inaequalis* (Cke.) Wint. have been under investigation at Wisconsin for some years, 10, 5, 18, and 6). The present paper reports a phase of these studies concerned with variation in the pathogen. This work (14) has dealt chiefly with types and magnitudes of variation. It is hoped that the results may facilitate further inquiry into the nature and causes of these phenomena.

STUDIES OF PHYSIOLOGY

Materials

Since 1929 monoconidial cultures have been isolated by the method described by Keitt (9) from scabby apples obtained from several localities in Wisconsin and from Oregon, Michigan, and New York. These isolates were then placed on malt-agar slants and transfers made at frequent intervals for stock cultures, which usually were kept in an ice-box at 8° C.[3] The malt agar used throughout the cultural studies, unless otherwise noted, consisted of 25 grams of Trommer's malt extract, 17 grams of agar, and one liter of distilled water. It was sterilized at 15 pounds' pressure for 20 minutes. All cultural comparisons were made with material incubated in dark control chambers.

These monoconidial isolations were numbered in the order in which they were isolated. Cultures from Wisconsin apples were Nos. 1–2 Dudley, 3–14 and 23 variety unknown, 15 Winter Banana, 16 Northwestern Greening, 17, 20 and 36 Fameuse, 21 McIntosh, 22 Delicious; from Oregon 18, 19, 33 Yellow Newtown, 34 seedling, 35 Northern Spy; from Michigan 24 Northern Spy, 25 Steel Red, 26 McIntosh; from New York 27 McIntosh, 28 Rome Beauty, 29 Fall Pippin, 30 Fameuse, 31 Northern Spy, and 32 Cortland.

Colony Differences

Colony Characters. No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer is particularly grateful to Dr. G. W. Keitt, under whose advice this work has been pursued.

[3] The temperature notations throughout this contribution are in degrees Centigrade.

could easily be recognized. Monoconidial reisolations from the same culture were very uniform in colony characters. The cultures studied could be differentiated by such characters as: margin of colony, size of colony, color, and the amount of aerial mycelium. Many series of plate cultures were observed and the above characters were constant for a given culture under the same environment, with the exception of occasional sudden changes, which will be discussed later. Figure 1 shows some of the characteristic differences that distinguish the different cultures.

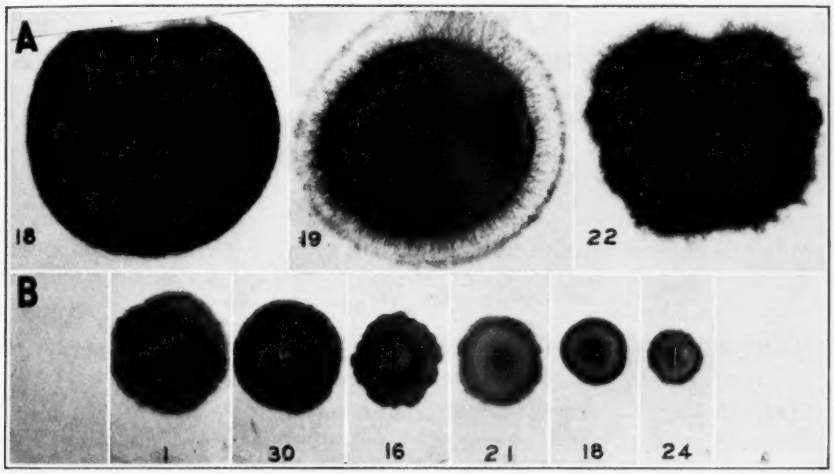

FIG. 1. Monoconidial cultures of *Venturia inaequalis* grown on malt-extract agar in Petri dishes at 21° C. The numbers indicate the different cultures. A. Grown for 48 days, note variations in margins. The colonies were grown in duplicate as indicated by the flattening at the top of the figure. B. Grown for 35 days.

Differences in colony margin were greatest when the cultures were grown under optimal conditions and they increased with the age of the colonies. On malt-extract agar the colony margins of most cultures were entire at temperatures above or below the optimum (16–22°). Marginal differences of 35-day-old colonies (Fig. 1, B) were not so great as those of the 48-day-old colonies shown in figure 1, A, where entire, filamentous, and lobate margins were very marked.

The several cultures grown under uniform conditions differed greatly in the size of the colonies produced in a given length of time. (Fig. 1, B.) Rate of growth is considered more fully under physiologic studies. In color the colonies of different cultures varied from a light mouse gray to a dark olive gray. The color is largely dependent on the amount of aerial mycelium, which varies with the medium and the culture. Wiesmann (17) has described similar differences in colony types of five isolations of *Venturia inaequalis*.

Spore Production. Certain cultures of *Venturia inaequalis* produced conidia more abundantly than others under uniform conditions. Records were made of the production of conidia and the initiation of perithecia in stock cultures of certain cultures grown on malt-extract agar in test tubes kept in an ice-box at 8°. The stock cultures were in duplicate and in each case both tubes conformed closely in the number of spores and perithecia when small portions of the agar and mycelium were examined under the microscope. No mature perithecia have been found in these cultures. In one series cultures were observed after 2 months and in others after 9 and 12 months. All degrees of spore production were found. The cultures were quite constant in spore production under the conditions of the experiment. As might be expected, the older cultures in certain cases showed fewer conidia and more perithecia. Examinations of cultures grown on malt-extract agar during two temperature series have shown more abundant production of conidia at the temperatures from 8 to 16° than at 20° or above. Certain cultures produced some spores even at the higher temperatures, while others were almost free of spores at temperatures above 20°.

Physiologic Differences

Studies of physiologic differences between cultures of *Venturia inaequalis* included: (a) effect of source of isolation, temperature, hydrogen-ion concentration, and nitrogen source on the rate of growth; (b) differences in tolerance to toxic materials; and (c) stability in culture.

Differences in Rate of Growth

Differences in growth rate were easily noticeable when several cultures were grown in Petri dishes under standard conditions. Duplicate series, after 35 days on malt-extract agar at 21°, gave consistent results and revealed that the average diameter of the fastest growing culture was 40 per cent larger than that of the slowest growing culture.

Relation of Source of Isolation. With the object of finding whether any relation existed between the rate of growth and the apple variety from which the cultures were isolated or the locality from which they came, an experiment was set up using 13 cultures isolated from 4 varieties of apple secured from 4 States. The liquid medium used for the experiment consisted of the following ingredients in the stated concentrations: M/4 dextrose, M/5 KNO_3, M/20 KH_2PO_4, M/100 $MgSO_4$, and a trace of $FePO_4$. This solution in 10 cc. amounts was placed in 50 cc. flasks and steamed 1 hour on each of 2 successive days. Four flasks were planted with small equal portions of mycelium from each of the 13 cultures and were then kept at a constant temperature of 21° for 45 days. The mycelium was transferred to filter papers on a Büchner funnel and washed with distilled

water. After drying at 65° for 22 hours each filter paper, with the contents, was weighed.

In table 1 the cultures are arranged according to their rate of growth. Culture 30 from New York made the best growth while culture 27 from

TABLE 1.—*Relation of source of monoconidial cultures to rate of growth, production of conidia and perithecia in culture, and pathogenicity*

Culture number	Source of culture	Dry weight of mycelium[a]	Abundance of conidia[b]	Abundance of perithecia[b]	Reaction on Y. Transparent[c]
		Mgm.			
30	Fameuse N. Y.	93	+++	+	No infection
26	McIntosh Mich.	87	+	++	Infection
35	Northern Spy Ore.	68	+	+	.....
33	Yellow Newtown Ore.	67	++	+++	No infection
31	Northern Spy Ore.	65	+++	+	.....
21	McIntosh Wis.	58	+	+	Infection
18	Yellow Newtown Ore.	56	++	++	No infection
20	Fameuse Wis.	53	+	+	.....
17	Fameuse Wis.	49	+++	+	No infection
36	Fameuse Wis.	42	+++	++	.....
19	Yellow Newtown Ore.	38	+++	+	Infection
24	Northern Spy Mich.	36	+	+	Infection
27	McIntosh N. Y.	24	+++	+	.....

[a] Dry weight of mycelium in mgm. after 45 days in synthetic solution at 21°.

[b] Relative abundance of conidia and perithecia present after 2 months on malt-extract agar at 8°; +++ = abundant; ++ = moderate; + = sparse.

[c] Results of inoculations on Yellow Transparent trees in the greenhouse.

New York made the least. The 3 cultures isolated from Yellow Newtown apples from Oregon showed much variation. Culture 33 made a growth almost twice that of 19. The abundance of conidia and perthecia and ability to infect leaves of Yellow Transparent have been added to the table as they give further evidence that variations in cultures isolated from a single variety and locality are as great as between cultures from widely separated localities or different varieties. Cultures 18 and 19 were isolated at the same time from the same collection of apples, yet their characteristics are quite different. For instance, of the three isolations from Yellow New-

town apples from Oregon, cultures 33 and 18 failed to infect Yellow Transparent leaves, while culture 19 succeeded. Culture 33 initiated abundant perithecia; 18, a moderate number; and 19, only a few. While the number of cultures studied in this experiment was not large, the results indicate wide variation between cultures from a given locality and variety.

Wiesmann (17) found variations in growth rate, color, and shape of the mycelium when pure cultures of *Venturia inaequalis*, isolated from different apple varieties in the same locality, were grown in dilute pear and grape juice media. From pure culture studies he concluded that isolations made from conidia taken from infected twigs of a given host did not differ from isolations of conidia from leaves of the same host. His results agree with the findings of the present writer in indicating the presence of several widely different strains of *V. inaequalis* in one locality.

Effect of Temperature. Keitt and Jones (10) give 16° to 22° as the optimal temperature for ascospore germination and germ-tube growth. This temperature range also was found favorable for the growth of the mycelium on agar media and for leaf infection of the host plants. Studies made by the present writer to determine whether cultures had different temperature requirements revealed that the isolations considered had the same optimal temperature for growth but at this temperature the cultures varied greatly in their rates of growth.

Eight cultures were used for a study of the effects of temperature on growth rate. For the first series malt-extract agar was used with the addition of 2.4 grams of $MgSO_4$ and 6.8 grams of KH_2PO_4 per liter to help buffer the medium. Petri dishes containing 25 cc. of this medium were inoculated at their centers with small portions of mycelium. The dishes were then bound with adhesive tape to prevent drying at the higher temperatures. Five plates of each of six cultures (1, 16, 18, 21, 24, and 30) were placed at the following temperatures for 5 weeks: 7, 17, 21, and 27°, variable 1°. At the temperatures considered, culture 24 consistently grew more slowly than any other; no culture, however, grew consistently faster than the rest. At 21° culture 1 produced the largest colonies, at 27° colonies of culture 30 were the largest, and at 7° cultures 16 and 21 grew faster than the others. Each culture grew most rapidly at 21°, which is near the optimal temperature for mycelial growth of *Venturia inaequalis*. The average colony diameters at 21° were: Culture No. 1, 50 mm.; No. 16, 37 mm.; No. 18, 26 mm.; No. 21, 35 mm.; No. 24, 26 mm.; and No. 30, 45 mm.

A second series differed from the first by the omission of $MgSO_4$ and KH_2PO_4 from the medium and by the substitution of wide rubber bands for the tape to prevent drying. This series included cultures 19, 22, 24, and 30 at each of the following temperatures: 4, 8, 12, 16, 20, 24, 28, and

32°, with a variability of 1°. The replications gave consistent results and are averaged in table 2, which shows the final diameters of the colonies of each culture at the different temperatures at the end of 5 weeks.

TABLE 2.—*Effect of temperature on the diameter of colonies of four monoconidial cultures of Venturia inaequalis on malt-extract agar*

Temp. ° C.	Diameter of colony in mm. after 5 weeks for stated cultures			
	19	22	24	30
4	6	8	4	14
8	12	10	9	21
12	17	18	12	30
16	17	18	14	34
20	20	22	15	44
24	19	16	12	35
28	10	1	1	11
32	0	0	0	0

In the second series all four cultures grew throughout a temperature range from 4° to 28°, but none grew at 32°. Culture 30 produced the largest and culture 24, the smallest colonies at each temperature. Cultures 22 and 19 showed similar growth rates at the lower temperatures but 22 grew slower at the higher temperatures than 19. Although the optimal temperature for growth on malt agar for all the cultures studied lies near 21°, yet, at this temperature, certain cultures grew twice as rapidly as others. The relative rates of growth of the cultures change at temperatures above and below the optimum.

Effect of Hydrogen-ion Concentration. Three series of experiments have been made to find whether the cultures have different H-ion relations. In the first series two cultures were used, 18 that had given infection on Wealthy and Fameuse, but none on Yellow Transparent leaves, and 21, which had given good infection on all 3 varieties. The cultures were grown in a modified Czapek's solution to which a mixed buffer solution was added, the pH being varied by the addition of increasing amounts of sodium hydroxide as described by MacInnes (12). Flasks containing 56 cc. of the medium were sterilized and planted with small equal portions of mycelium. Culture 21 was duplicated in a series exactly the same as the first, except that the flasks were planted with spore suspensions. After 47 days, at 21°, the mycelium was removed from solution to weighed filter papers in a Büchner funnel, washed with distilled water, and dried for 26 hours at 65°. (Table 3).

Another pH series was set up using malt-extract solution inoculated with 5 different cultures. Cultures 24 and 21 gave infection on Yellow Transparent leaves in 1930, while the other 3, cultures 16, 18, and 30, gave no infection on Yellow Transparent leaves but did infect those of Wealthy and Fameuse. The nutrient solution was made up in one lot as follows:

Trommer's malt extract	125 grams
KH_2PO_4	34 grams
$MgSO_4$	12 grams
H_2O	5000 cc.

This solution was divided into 5 lots and normal HCl or NaOH was added to bring them to the proper pH values. The solution in 50 cc. amounts was then placed in 250-cc. flasks and sterilized at 12 pounds' pressure for 30 minutes. Five flasks were planted with each culture for

TABLE 3.—*The effect of hydrogen-ion concentration on the growth of certain monoconidial cultures of Venturia inaequalis*

Culture Series 1	Average dry weight of mycelium in mgm. in Czapek's solution of varying pH after 47 days at 21°					
	2.6	3.6	5.0	6.6	8.6	9.4
No. 18	1.8	35.3	61.6	12.6	8.0	5.6
No. 21	3.0	17.0	22.3	10.6	9.5	8.3
No. 21[a]	2.5	21.5	33.6	9.0	0.0	0.0
Series 2	**Average dry weight of mycelium in mgm. in malt extract of varying pH after 41 days at 21°**					
	3.3	3.8	4.8	5.4	6.0	
No. 16	103	284	.....	291	212	
No. 18	80	168	182	209	145	
No. 21	47	97	113	233	135	
No. 24	70	72	.....	105	92	
No. 30	100	167	.....	158	147	
Series 3	**Average diameter increase in mm. of colonies on malt-extract agar of varying pH after 35 days at 20°**					
	3.4	4.8	5.4	6.4	7.4	
No. 15	12	17	18	17	15	
No. 18	24	24	27	26	23	
No. 19	13	20	21	16	17	
No. 22	14	21	23	19	16	
No. 24	16	22	22	17	14	
No. 30	43	37	35	31	24	
No. 33	29	41	39	31	25	

[a] Started with spores instead of mycelium.

each pH. Mycelium on malt-agar slants was cut up and introduced with about 2 cc. of water into the flasks, which were kept at 21° for 41 days. Results were taken by weighing the mycelium as in the previous series. (Table 3).

A third hydrogen-ion series was set up, using a buffered malt-extract agar as the medium, so that the growth rate might be followed throughout the experiment. The buffer materials were added to the agar in the following amounts:

Citric acid	8.40 grams	Agar	80.00 grams
Glycine	3.04 grams	H_2O	3500.00 cc.
KH_2PO_4	3.63 grams		

These materials were dissolved and then divided into 10 lots of 340 cc. and brought to the required pH by adding approximately normal NaOH in suitable amounts, so that the range extended from pH 3.4 to 7.4. The malt extract (100 grams in 250 cc. of water) was sterilized separately and added to the buffer-agar mixture after sterilization in 25 cc. amounts with enough sterile water to bring the total volume of each flask to 400 cc. Sixteen Petri dishes were then poured from each flask so that every dish contained about 25 cc. of the medium. Samples from each flask were saved and the pH determined by a quinhydrone electrode.

The seven cultures used in this study were placed on the medium as 4 mm. discs cut from 10-day-old plate cultures with a stiff wire loop. Two discs of the same culture were placed in each Petri dish. Cultures 18, 19, and 24 were repeated 6 times at each pH, while 15, 22, and 33 were repeated 4 times. Culture 30 had only 2 colonies at each pH, except at pH 3.4 and pH 5.4, where 4 colonies were grown. The diameters of the colonies were measured every 7th day for a period of 5 weeks. The results are shown in table 3, which gives the final colony diameters of the seven cultures at each hydrogen-ion concentration.

The medium was tested at the end of the experiment for any change in pH. A slight increase in pH was found throughout the entire range, except the medium with an initial pH of 7.4, which had changed very little. The medium on which culture 18 had grown showed a greater change in pH throughout the series than the corresponding medium on which the slower growing culture 15 had developed. Also it was observed that the greatest change in hydrogen-ion concentration was found at the most favorable pH for growth of the cultures, which indicates a direct correlation between the amount of fungus growth and the change in pH.

Venturia inaequalis grew in a modified Czapek's solution throughout a range from pH 2.6 to 9.4. Conidia of culture 21 germinated in solutions ranging from pH 2.6 to 6.6 but did not germinate in solutions of pH 8.6 or

9.4. When grown in malt-extract solution at 20° cultures 16, 21, 18, and 24 grew better at pH 5.4 than at pH 4.8 or 6.0. Culture 30 grew slightly better at pH 3.8 than at pH 3.3 or 5.4. When grown on malt-extract agar at 20° cultures 19, 22, and 24 grew best at pH 5.4, while cultures 18 and 15 made slightly better growth at pH 5.6. Culture 33 grew best at pH 4.8 and 30 at pH 3.4. Certain cultures have been included in each of the series, and the results obtained in the 2 series check favorably.

The optimal hydrogen-ion concentration for all the cultures studied lies between pH 4.8 and 5.6 with the exception of culture 30, which grew better at pH 3.4 than at the higher hydrogen-ion concentrations. Even though certain cultures have the same optimal hydrogen-ion concentration for growth, there is much difference in the rate of growth of the cultures at that concentration.

Wiesmann (17) found that "races" of *Venturia inaequalis* behaved differently on yeast extract at the neutral point and with various amounts of tartaric acid added. His Virginia "form" grew best on the neutral medium, while Grossherzog, Wellington, and Boiken forms grew best on solutions made acid with 0.1, 0.2, and 0.3 per cent concentrations of tartaric acid, respectively.

Effect of Nitrogen Source. The source of nitrogen has a marked influence on the growth of *Venturia inaequalis*. In order to determine whether cultures varied in their response to nitrogen source, they were grown on nutrient solutions containing nitrogen supplied by ammonium sulphate, potassium nitrate, and calcium nitrate, respectively. Two series were run in which the following materials were used in amounts to give the indicated dilutions: Dextrose M/3, KNO_3 M/5 KH_2PO_4 M/20, $MgSO_4$ M/100, $FePO_4$ trace. This solution was used in 50 cc. portions in 250 cc. flasks with $Ca(NO_3)_2$ M/5 or $(NH_4)_2SO_4$ M/5 substituted for KNO_3, as indicated in table 4. The flasks were planted with small portions of mycelium in the first series, and with spore suspensions in the second series, then kept at 20° for 45 days. The results are given in table 4 as the oven dry weight of the mycelium (dried 24 hours at 80°).

Cultures 18 and 21 both grew best when $(NH_4)_2SO_4$ was used as the source of nitrogen and both made about the same amount of growth on this solution. Culture 18 made better growth in the $Ca(NO_3)_2$ than in the KNO_3 solution, while culture 21 grew much better in the KNO_3 solution. Cultures 17, 19, 24, and 30 reacted like 21 on these solutions. No other culture has been found that reacts like 18 in preferring $Ca(NO_3)_2$ to KNO_3.

DIFFERENCES IN TOLERANCE TO TOXIC MATERIALS

Certain chemicals used in fungicides are toxic to *Venturia inaequalis* and when placed in malt-agar media in sufficient amounts will inhibit

growth of the fungus. The following experiments were made in order to determine whether the percentage of the toxic material necessary to inhibit growth would vary with the different strains of the fungus.[4]

The methods suggested by Schmitz (15) for toximetric studies of wood preservatives have been followed in these experiments and will be given only a brief description. Malt-extract agar was used as the nutrient medium and the chemicals were sterilized in part of the water and mixed with the agar just before being poured into the Petri dishes. Approximately 25 cc. of nutrient-chemical mixture was placed in each dish and planted with two 4-mm. discs of mycelium from 10-day-old cultures on malt-extract agar. The test plates were incubated for 3 weeks at 20°. Where no growth occurred on the plates or discs after 3 weeks the discs were transferred to standard malt-agar slants at 20° to determine whether the fungus was dead or alive. Then, if no growth occurred in 3 weeks, the fungus was considered dead.

Only materials employed as commercial sprays were tested. Sulphur dust and liquid lime-sulphur were tried in preliminary tests but were not satisfactory. Paris green and especially copper sulphate were much better adapted to this type of study and the results obtained with these materials will be presented.

Certain cultures were found to be much more tolerant to copper sulphate than others (table 5). Less difference in relative tolerance was found when Paris green was employed as a toxic agent.

STABILITY IN CULTURE

During the 3 years that the writer has had monoconidial cultures of *Venturia inaequalis* under observation, several have shown sudden changes in color, rate of growth, and spore production. Usually these changed characters were retained through asexual propagation. In other cases the cultures continued to change but seldom reverted to the original type. Other cultures were apparently more stable as no changes were observed to occur in them. The variations usually appeared as fan-shape sectors starting at or near the center of the colony.

As far as the writer has observed, sectoring in *Venturia inaequalis* is usually associated with loss of spore production by the sectors. The sectors usually grow more rapidly than the original colony and differ slightly in color. By placing the cultures at temperatures of 12 to 16° the sectors usually produced a few spores, which sometimes appeared abnormal because of odd shapes and the habit of germinating before being cut off. Culture 19 is shown in figure 2 with a sterile sector. The original colony was sporu-

[4] With the permission of Dr. G. W. Keitt, these data are taken from studies of the toxicity of various substances to *V. inaequalis*, in which the writer assisted.

lating freely (Fig. 2, C), while the sector was producing only a very few conidia (Fig 2, D) after 20 days at 12°.

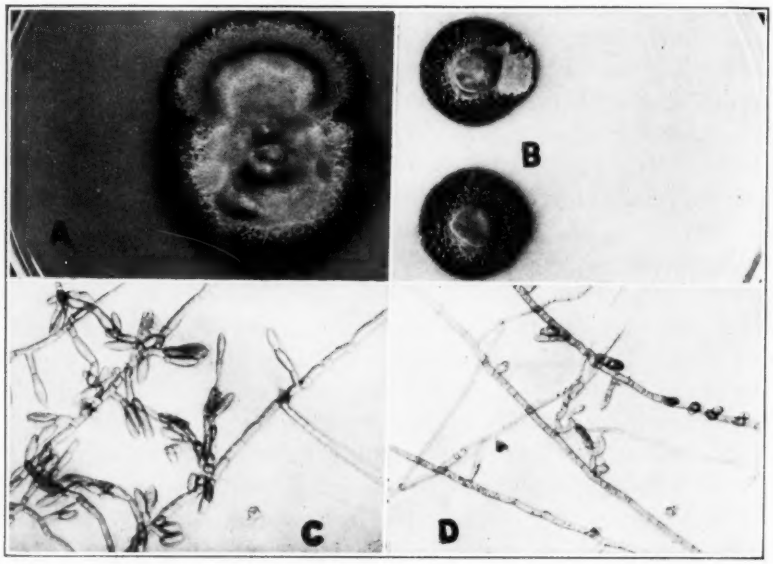

FIG. 2. Sterile sectors of culture 19 grown on malt-extract agar in Petri dishes. A. Three weeks at 20° C. followed by 20 days at 12° C. B. Found in pH series at pH 3.4 after 35 days at 20° C. C. Sporulating margin of the lower part of colony shown in A. D. Sterile margin of sector shown in A. C. and D. ×160.

When portions of these sterile sectors were transferred to Petri dishes and compared with the original culture the colonies were usually larger, more dense, and of slightly different color. Sometimes these sterile cultures produced other sectors differing in color and rate of growth. None have been observed to regain their ability to produce conidia abundantly.

TABLE 4.—*Effect of nitrogen source on monoconidial cultures of Venturia inequalis in nutrient[a] solution for 45 days at 20° C.*

Nitrogen source	Series	Culture 18		Culture 21	
		Flasks used	Average weight of mycelium	Flasks used	Average weight of mycelium
		No.	*Mgm.*	*No.*	*Mgm.*
KNO_3	First	3	31	3	42
"	Second	15	31	15	45
$Ca(NO_3)_2$	First	2	59	5	18
"	Second	5	44	5	11
$(NH_4)_2SO_4$	Second	5	100	5	102

[a] The composition of the medium used is given in the text.

Aderhold (1) observed that the mycelium of *Venturia inaequalis*, in culture, undergoes a gradual differentiation into two types. In one type he found sparse mycelial growth and abundant sporulation; in the other, an abundant mycelium was produced and only a few spores. He considered that lack of nutrient was a likely cause. He also reported that towards autumn the richness of spores diminished. In winter he found little conidial production but much mycelium. However, he grew his cultures at 25°, while the writer found production of conidia most abundant at temperatures below 20°. Stock cultures on malt-extract agar kept at 8° in an ice-box were still sporulating freely after 3 years. As shown earlier in this paper, certain strains always produce spores more abundantly than others.

Besides the sectoring colonies mentioned, other indications of instability in culture have been noted. Culture 16, used in the inoculation series of 1930, later produced so few conidia that its use in this work had to be discontinued. Culture 21 gave good infection during 1930 on 3 varieties but, in 1931, no infection was obtained with conidia of this culture on these or

TABLE 5.—*Percentage of copper sulphate or Paris green in the culture medium tolerated by different monoconidial cultures of Venturia inaequalis*

Culture used	Replications averaged	Per cent of $CuSO_4$ in medium:					
		0.06	0.08	0.10	0.12	0.14	0.16
	No.						
17	4	++[a]	+	−	−	−	−
30	4	++	++	+	+	+	−
22	12	++	++	+	+	−	−
15	6	++	+	−	−	−	−
18	6	++	++	++	++	+	−
24	8	+	+	−	−	−	−
33	4	++	++	+	−	−	−
		Per cent of Paris green in medium:					
		0.10	0.18	0.22	0.26	0.30	0.34
17	4	++	+	+	+	−	−
30	4	++	++	++	++	++	++
22	12	++	++	+	+	+	+
15	6	++	++	+	+	+	−
18	6	++	+	+	+	−	−
24	6	++	+	+	+	+	−
33	4	++	++	++	+	+	+

[a] ++ = measurable growth on the toxic medium in three weeks; + = no measurable growth on the toxic medium but growth when transferred to malt-agar slants after three weeks on the toxic medium; − = fungus killed, no growth when transferred to malt-agar slants after three weeks on the toxic medium.

other varieties. This culture, apparently, was no longer pathogenic. A possible explanation of such changes would be the chance transfer of portions of sterile or nonpathogenic sectors arising in stock cultures where their presence would not be readily noticed.

Since certain of the cultures appeared to be changing, reisolations were made of several of the cultures to ascertain whether single conidia of the same culture would produce similar colonies or colonies differing in certain characters. Eighteen single conidia of culture 22 and 6 single conidia of culture 24 were isolated in the manner described earlier and allowed to develop on malt-extract-agar slants. After 10 days transfers were made to Petri dishes containing malt-extract agar. Two small portions of mycelium developed from single conidia were then placed in the Petri dishes. These cultures were kept at 20° for three weeks and then compared in general appearance, size of colony, and color. All single-spore colonies from each culture were consistent in all the above characters.

STUDIES OF PATHOGENICITY

Conidial inoculations were made on 20 commercial apple varieties and 6 closely related genera to determine the different infection capabilities of certain monoconidial cultures of *Venturia inaequalis*.

METHODS AND MATERIALS

Spore Production. The spores used for inoculations were produced on cheesecloth wicks in a nutrient solution. Cheesecloth was cut into pieces about 5 by 10 cm. and placed in test tubes so that it formed a lining for the tubes. About 3 cc. of nutrient solution (25 grams of Trommer's malt extract in one liter of water) was introduced into each tube. The tubes were then sterilized in the autoclave at 15 pounds' pressure for 20 minutes. About 2 weeks before the spores were needed, the tubes were inoculated by placing bits of mycelium from malt-agar slants on the cheesecloth wicks and spreading it over the surface of the wicks with a sterile needle. Care was taken not to introduce any more agar than necessary. After inoculation the tubes were kept at 20° for a week and then placed at 14 to 16° until needed, as the lower temperature favored spore production.

When a spore suspension was needed for inoculations the cheesecloth wicks were removed from the tubes and placed in the top part of a Petri dish fixed in an upright position and standing inside the lower half of the dish. Distilled water was used in an atomizer to wash the spores from the wicks into the dish. The abundance of spores varied with different strains, so more water was added to the heavily sporulating cultures until the spore suspensions of all were of about the same concentration. All suspensions were adjusted to an average of 10 spores per low-power field of the microscope. The trees were inoculated by spraying them with the spore suspen-

sion by means of an atomizer. Both the upper and lower surfaces of the leaves were covered as evenly as feasible.

Care of the Trees. One- and 2-year-old potted trees limited to 2 or 3 twigs each were used for these studies. All the trees were, so far as possible, kept in the same greenhouse and under the same environmental conditions. Trees with the same amount of shoot growth were used for a given series. After inoculation, the trees were placed in the moist chamber, devised by Keitt (10), at a temperature of 16° to 20° where they remained for 48 hours, unless otherwise stated.

When removed from the moist chamber the trees were placed in the greenhouse. After 3 weeks had been allowed for the disease to develop, records were made of the scab lesions on each infected leaf. The maximum number of lesions recorded on any one leaf was 100, regardless of any greater amount of infection, as counts could not be made with satisfactory accuracy if more than 100 lesions were present, and a quantitative record above 100 would add little value to the data.

INFECTION STUDIES ON CERTAIN SPECIES RELATED TO APPLE

Aderhold (2) reported *Fusicladium dendriticum* on the following Pyrus species: *P. spectabilis* Ait., *P. kaido* Sieb., *P. floribunda* Sieb., *P. baccata* L., *P. prunifolia* Willd., *P. rivularis* Hook., and *P. dioica* Mnch. In 1903 Aderhold (4) recorded cultural experiments that showed *Fusicladium crataegi* Adh. to be distinctly different from *F. dendriticum*. Aderhold concluded that the fungus on Crataegus was entirely distinct from that on apple and that cross infections were unlikely. He also described experiments by which he determined that *F. orbiculatum* Denn. on *Sorbus torminalis*, which is morphologically closely related to *F. dendriticum*, cannot infect apple.

Very little recent work has been reported on this phase of the apple-scab problem. In order to extend the knowledge on this phase and to determine the infection capabilities of different cultures on the several hosts a series of experiments was started in which 29 species representing 6 genera closely related to the apple were inoculated with 6 monoconidial cultures of *Venturia inaequalis*.

Results of Inoculations on 6 Genera. During the spring of 1931 the following genera were inoculated as potted trees in the greenhouse; 13 species of Malus, 9 of Crataegus, 3 of Sorbus, 2 of Amelanchier, 1 of Aronia, and 1 of Cotoneaster. These plants were inoculated with cultures 15, 17, 18, 19, 24, and 30. Two or more trees of each species were used in each series. All these plants were obtained from the Kelsey-Highlands Nursery, East Boxford, Massachusetts. Only 9 of the 13 Malus species inoculated were infected by the cultures studied, the other 4 remained entirely resistant to all

6 of the cultures. None of the 5 genera, other than Malus, showed any signs of infection. The results of this work are in accord with those of Aderhold in that infection by *Venturia inaequalis* occurred only on hosts within the genus Malus.

Results of Inoculations on Malus Species. The Malus species studied showed some interesting reactions to the different cultures used for inoculations (Table 6). The 9 species infected by 1 or more of the cultures can

TABLE 6.—*Results of infection experiments with monoconidial cultures of Venturia inaequalis on certain Malus species*

Species of Malus inoculated	Culture number						Cultures used	Cultures infecting
	15	17	18	19	24	30		
							No.	*No.*
niedzwetzkyana	+[a]	+	+	+	+	+	6	6
ioensis	+	...	+	...	+	+	4	4
robusta	...	...	+	...	+	+	3	3
baccata	+	0	+	+	0	+	6	4
sieboldii	+	+	0	0	0	0	6	2
toringoides	+	+	...	+	...	0	4	3
coronaria	...	...	+	...	0	+	3	2
sargentii	...	...	+	0	0	0	4	1
tschonoskii	+	...	0	0	0	0	5	1
theifera	0	0	0	0	0	0	6	0
angustifolia	0	0	0	0	0	0	6	0
floribunda	0	0	0	0	0	0	6	0
arnoldiana	0	0	0	0	0	...	5	0

[a] This experiment was conducted on potted trees in the greenhouse; + = infection; 0 = no infection.

be placed in 2 groups: those infected by all of the cultures, and those infected by some and resistant to others. Those species infected by all of the cultures include: *M. niedzwetzkyana* infected by all 6 cultures, *M. ioensis* infected by 4, and *M. robusta* infected by 3. *M. baccata, M. sieboldii, M. toringoides, M. coronaria, M. sargentii,* and *M. tschonoskii* were each infected by 1 or more of the cultures and resistant to others. The 4 species entirely resistant to all 6 cultures were: *M. theifera, M. angustifolia, M. floribunda,* and *M. arnoldiana.* A study of a larger number of cultures may reveal some that are capable of infecting these other Malus species.[5]

[5] The source and identity of the species here concerned as furnished by Harlan P. Kelsey, Jr., is as follows: *Malus niedzwetzkyana,* obtained from grafts from original stock from Arnold Arboretum; *M. ioensis,* grafts from A. A.; *M. robusta,* grafts and buds from A. A.; *M. theifera,* buds and grafts from A. A.; *M. arnoldiana,* buds and grafts from A. A.; *M. baccata,* buds from A. A.; *M. toringoides,* buds from A. A.;

Reisolations of the fungus were made from all the species infected, except *Malus tschonoskii,* and the cultural characters in each case appeared the same as in the original culture. When spores of these reisolated cultures were used to inoculate varieties of *Malus malus,* good infection was obtained.

Plant Variations. In working with these Malus species some variations in the plants were noted. The plants were not so uniform as the commercial apple varieties used for infection studies. Ten trees of each species were potted and were usually similar in morphological appearance but in each of 4 species certain trees showed different pathogenic reactions. One example will suffice to indicate the kind and amount of variation found. Ten trees of *Malus baccata* were inoculated with 6 different cultures and the results obtained are shown in table 7. For convenience the trees are given refer-

TABLE 7.—*Variation in resistance to infection as shown by ten trees of Malus baccata inoculated with monoconidial cultures of Venturia inaequalis*

Culture number	Individual trees									
	A	B	C	D	E	F	G	H	I	J
15					0				+	
17			0				0			
18	+			0				0	0	+
19	+									+
24	0				0	0	0	0		0
30		+		0						

ence letters from A to J. Some of the trees were infected by a given culture, while other trees were not infected by the same culture. Trees A and J were infected by cultures 18 and 19 but not by 24, while trees C, D, E, F, G, and H were not infected by any of the cultures. Tree I was infected by culture 15, while tree E was not nor was I infected by culture 18, which infected trees A and J. Culture 30 was able to infect tree B but did not infect tree D. *Malus robusta, M. sargentii,* and *M. coronaria* showed similar variations in susceptibility. It is apparent that in these cases both the suscept and the pathogen are quite variable. Such variations as are shown in table 7 between cultures 18 and 24 or 15 cannot be disregarded. These differences in the infection capabilities of cultures on certain Malus species, when considered with similar differences on the less variable apple varieties, have been interpreted as showing the variability between certain cultures of *Venturia inaequalis.*

M. sargentii, seeds from A. A.; *M. angustifolia,* grafts from A. A.; *M. sieboldii,* seeds from A. A.; *M. coronaria,* grafts from Chase Nursery Company, Chase, Alabama; *M. tschonoskii,* seeds from Japan; and *M. floribunda,* grafts from Chase Nursery Company.

INFECTION STUDIES ON CERTAIN APPLE VARIETIES

Experiments of 1930. During the spring of 1930 Yellow Transparent, Wealthy, and Fameuse trees were inoculated with conidia of 7 different cultures in 3 series of experiments. Yellow Transparent trees were not infected by 3 of the cultures but were heavily infected by the other 4. The Wealthy and Fameuse trees were infected by all of the cultures tried.

The data obtained are given (Table 8) for representative series. The 3

TABLE 8.—*Results of infection experiments with monoconidial cultures of Venturia inaequalis on three apple varieties in 1930*

Inoculation date and culture	Maximum number of lesions per leaf[a]		
	Yellow Transparent	Fameuse	Wealthy
April 1			
Wis. 15	0	31–68	5–19
Ore. 19	100	100	15–96
Wis. 21	100	30–100	28–100
Wis. 22	9–100	1–14	7–29
April 11			
Ore. 18	0	18–26	.....
Ore. 19	100	100	100
Wis. 21	50–100	20–27	31–100
Wis. 22	25–100	46–60	27–100
Mich. 24	100	100	100
April 25			
Wis. 16	0	100	100
Ore. 18	0	52–83	13–70
Wis. 21	23–36	18–43	19–36
Wis. 22	28–49	38–46	20–42
Mich. 24	100	40–64	53–100

[a] Four shoots on two or more trees were inoculated with each strain.

cultures (15, 16, and 18), which did not infect Yellow Transparent, were consistent in never producing lesions on leaves of this variety. Cultures 19, 21, and 24 produced abundant infection on all 3 varieties but usually showed more lesions on the Yellow Transparent than on the Fameuse or Wealthy trees. Culture 22 also infected all 3 varieties but not so heavily as the other 3 cultures.

Experiments of 1931. In the spring of 1931 further studies of pathogenicity were made, which included 20 varieties of apples inoculated with 9 cultures of *Venturia inaequalis.* These studies were made in a manner similar to that of 1930, the main difference being the use of only 1 culture

in a series instead of 4 or 5, as used during the previous season. This change made it possible to treat a large number of varieties in a series without danger of mixing the inoculum while the trees were in the inoculation chambers. Most series were repeated at least once and in all cases the results were quite consistent. Where no infection is reported macroscopic scab lesions were not present on any of the leaves. When vigorous trees were used either all the shoots showed infection or none of them were infected, depending on the variety and culture used.

TABLE 9.—*Results of infection experiments with monoconidial cultures of Venturia inaequalis on certain apple varieties in 1931*

Variety inoculated	Culture tested								
	Wisconsin			Oregon			Michigan		N.Y.
	15	17	22	18	19	33	24	26	30
Yellow Transparent	0	0	+	0	+	0	+	0	0
McIntosh	0			0	+	0	+		0
Dudley	+			0	0		+		+
Hubbardston				+	0	0	+		+
Missouri Pippin	0	0	+	+	0	+	+		+
Fameuse	+	+	+	+	+		+	+	+
Wealthy	+	+	+	+	+		+		+
York Imperial				+					+
Haralson		+		+	+	+			+
Baldwin			+	+	+				+
Red Astrachan		+		+		+			+
Early Harvest		+		+		+			+
Ben Davis		+		+		+			+
Ingram		+		+		+			+
Pewaukee				+	+		+		+
Golden Delicious					+				+
Twenty Ounce				+	+				+
Price Sweet		+				+			+
August Strawberry				+		+			+
King							+		+

The results of the inoculation experiments on apple varieties are shown in table 9, which represents a total of over 600 twigs including duplicate series, inoculated with 9 monoconidial cultures during 1931. Since this study included 20 varieties inoculated with 9 different cultures in several series, the problem of presenting the detailed data was greatly increased. Therefore, only a summary is given.

The varieties studied may be divided into 2 groups according to their susceptibility. The first group is composed of those that were susceptible in some degree to all of the cultures with which they were inoculated. The

second group is composed of those varieties infected by some cultures but not by others. Of the 20 varieties studied, 15 were more or less susceptible to all the cultures with which they were inoculated. However, there was a wide difference in the degree of infection among these varieties. Certain varieties like Fameuse, Red Astrachan, and Early Harvest showed considerable infection with each of the cultures used. Other varieties, as, for example, Ingram and Haralson, were abundantly infected by some cultures and only sparsely infected by others.

The second group was composed of 5 varieties that were not infected by some of the cultures but were by others. Trees were designated free from infection when no macroscopic scab lesions were apparent 3 weeks after inoculation, when the records were taken, or thereafter as long as the trees were under observation. Culture 30 when used on Yellow Transparent trees sometimes caused flecks to appear on the leaves but these disappeared after a few days. Modified treatments in the inoculation chambers were tried in attempts to produce normal lesions and spores but without success. Therefore this variety has been considered free from infection by culture 30 under the conditions of the experiments.

Yellow Transparent, McIntosh, Dudley, Missouri Pippin, and Hubbardston Nonsuch were the varieties not infected by certain cultures. Table 9 shows the cultures that were able to infect each of these varieties. From these data it can be seen that some varieties are susceptible to certain cultures of the fungus from a given region but resistant to other cultures from the same region.

Table 9 shows clearly that the cultures differed in their infection capabilities on certain varieties. Culture 24 from Michigan was able to infect all varieties on which it was used and differed in this respect from all the other cultures studied. Culture N. Y. 30 differed from Ore. 18 by infecting Dudley. Ore. 19 differed from both N. Y. 30 and Ore. 18 because it was able to infect Yellow Transparent and McIntosh but not Missouri Pippin and Hubbardston Nonsuch, which were infected by 30 and 18. Wis. 17 differed from culture 18, 30, and 33 because it did not infect Missouri Pippin. Culture 15 from Wisconsin differs from 18 and 19 from Oregon by causing abundant infection on Dudley. The 1931 results confirmed those obtained in 1930 and further indicated strain specialization of *Venturia inaequalis*.

Wiesmann (17) has reported specialization in *Venturia inaequalis*. Although he obtained mostly negative results when leaves were inoculated with conidia from pure cultures, when conidia from a given host were used they were reported to be 2 to 4 times as virulent on leaves of the same variety as on those of other varieties. Most of his infection studies were made with cut leaves of 4 varieties: Wellington, Boiken, Virginia, and Gravenstein; and each variety was partly susceptible to infection by conidia from

the other 3 varieties. His report that none of these varieties was immune from conidia of any race might be explained by the fact that only 4 were studied and that the conidia were obtained from leaves and not from pure monoconidial culture and, therefore, might have contained more than one strain. In his infection studies the writer found that most of the varieties used were partly susceptible to all cultures, but 5 varieties were not infected by certain cultures, though susceptible to others.

STUDIES OF MORPHOLOGY

Besides the differences found in the cultural and infection studies certain cultures also showed slight morphologic differences. For this study the length of conidia was used as a criterion of culture differences. The conidia of *Venturia inaequalis* are rather variable in size, even when obtained from infected leaves. Aderhold (1) gave the length of conidia as 16 to 25 microns and the width as 6 to 10 microns. Most spores had a length of 18 to 22 microns and width of 8 to 10 microns. Wiesmann (17) compared the length of conidia taken from leaves of 4 apple varieties. By measuring 500 spores, each, from leaves of Wellington, Virginia, Gravenstein, and Boiken he found that the peak of curves drawn from the spore lengths fell at 24, 18.6, 20, and 21.2 microns, respectively. These results he concluded gave further proof that the scab strain on each of the above apple varieties was a "specialized race."

Conidial Length in Culture. In one part of the writer's study the spores were obtained from pure cultures grown under uniform conditions. The spores were produced on cheesecloth wicks in malt-extract solution, as described in the methods used during the study of pathogenicity. The conidia were mounted in glycerine and 300 spores from each of the 7 cultures were measured. By placing a stage micrometer under the high-power field of the microscope with a camera lucida attached, a scale divided into microns was drawn on white paper. Then, by replacing the stage micrometer with a slide of mounted spores, the length of the conidia could be measured directly in microns. A mechanical stage was employed so that no spores were measured twice.

The results of the measurements are shown in figure 3, A. It is easily seen that the typical spore length of 5 of the cultures falls in the range from 20 to 22 microns. Culture 19 produced smaller spores and its curve reached a peak at 18 microns. Culture 30 produced larger spores than any of the other cultures and its representative curve reached a peak at 27 microns, although many spores were much larger. Sixteen per cent of the spores of culture 30 and 11 per cent of the spores from cultures 15 were bicellular. The cultures also varied in spore shape. For example, cultures 18 and 19 produced spores with rounded ends in contrast to the pointed spores of the other cultures.

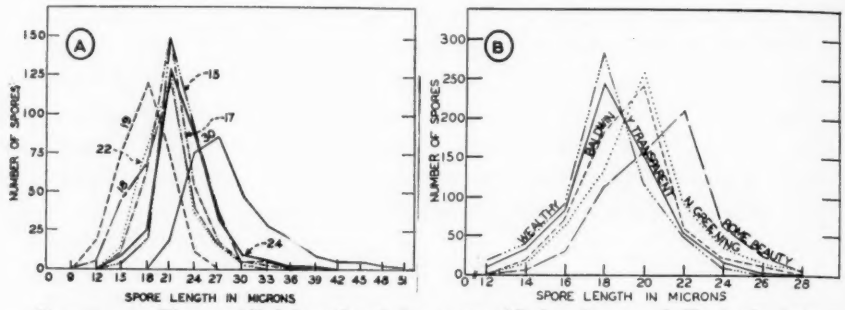

FIG. 3. A. The conidial length of 7 monoconidial cultures of *Venturia inaequalis* grown on malt-extract solution. Three hundred spores of each culture were measured under standardized conditions. B. The length of conidia of monoconidial culture 22 produced on Wealthy, Baldwin, Yellow Transparent, Northwestern Greening, and Rome Beauty trees in the greenhouse. Six hundred spores from each variety were measured under standardized conditions.

Conidial Length on Certain Apple Varieties. Preliminary study was made of spores washed from leaves of 3 varieties inoculated with culture 30 in the greenhouse during the infection studies of 1931. The interesting fact suggested by these observations was that spores produced on different varieties by the same culture varied in length. To study further the effect of host varieties on the length of conidia, several varieties were inoculated in the greenhouse with culture 22. Conidia from Wealthy, Yellow Transparent, Rome Beauty, Baldwin, and Northwestern Greening varieties were washed from the leaves and mounted in lactophenol solution for measuring. Two or more samples from different leaves were taken from each variety and 300 spores from each sample were measured. In each case, where spores were obtained from the ventral surface of the leaves, the different samples from the same variety were quite consistent. Spores taken from the dorsal surface of Baldwin leaves were shorter than those from the ventral surface. Two samples from each variety have been combined in figure 3, B, which shows the frequency curves obtained from the 600 spore measurements from each variety. These spores were all taken from the ventral surfaces of the leaves.

The modal length of conidia from Rome Beauty was 22 microns, representing 34.7 per cent of the spores. The mode for spores from Baldwin and Northwestern Greening was 20 microns, representing 40.4 and 43.2 per cent of the spores, respectively. The mode for Wealthy and Yellow Transparent was 18 microns, representing 40.4 and 46.3 per cent of the spores, respectively.

When culture 15 was used to infect Wealthy and Northwestern Greening the spore lengths were similar to those of culture 22, but 15 on Rome Beauty produced spores much shorter than those produced by 22 on this

variety. The modal conidial length of culture 22 on Rome Beauty was 22 microns and that of 15 was only 18 microns.

The present results indicate that different varieties varied the length of conidia of *Venturia inaequalis* when the varieties were infected by the same monoconidial culture. Moreover, on certain varieties different monoconidial cultures produced spores differing as much as 4 microns in their modal conidial length.

DISCUSSION

Origin of Strains. The major theories regarding the origin of physiologic forms of fungi are as follows: (a) adaptation, (b) mutation, and (c) hybridization.

No evidence has been found to indicate that adaptation to environment occasions more than temporary changes in monoconidial strains of *Venturia inaequalis.*

Mutations in parasitic fungi no doubt occur, and it is probable that new strains arise by this means. Sectoring has been noted in several monoconidial cultures of *Venturia inaequalis* but its nature has not been determined. Hansen and Smith (7) have presented proof that variations in *Botrytis cinerea* are due to the assortment of the nuclei and that this assortment is accomplished by the mechanisms of anastomosis and cell division.

The writer has noted hyphal fusions of *Venturia inaequalis* in culture. Besides the fusion of mycelial threads anastomosis of the germ tubes of conidia has been observed, often as many as 5 or 6 germ tubes being united. Frey (5) states that the cells of the mycelium are uninucleate and that each cell of the conidium is uninucleate. The nuclear behavior of *V. inaequalis* during anastomoses has not been studied, so the significance of the phenomenon in this fungus cannot be stated. However, until further study is made the possibility that variability may occur by this means must remain an open question.

Hybridization is known to occur in many fungi. The facts reported by Killian (11) and Frey (5) and confirmed by Wilson (18) show functional sex organs and suggest a true sexual process in *Venturia inaequalis.* Jones (8) reported the production of mature ascospores of *V. inaequalis* in culture after 5 months on oatmeal agar, but single-spore cultures were not used. Perithecia were initiated on several media by the writer when monoconidial cultures were used alone and in different combinations, but ascospores were not produced. It is not known whether *V. inaequalis* is homothallic or heterothallic. However, even though it be homothallic, it is possible that frequent matings between different strains might readily occur in nature and thus make possible the formation of new strains.

Monoconidial Strain Variation in Venturia inaequalis. Many workers [see bibliographies of Wallace (16) and Morris (13)] have listed certain

apple varieties as resistant or susceptible to apple scab. It is quite common to see trees of different varieties side by side, where one is heavily infected and the other is almost free of scab. Wallace (16) and others have pointed out many disagreements between reports of resistant and susceptible varieties. Aderhold (3) reported marked differences in the amount of disease on certain varieties in different seasons. Morris (13) states varieties relatively resistant in one locality may be quite susceptible in another.

It is quite likely that much of this disagreement is due to the prevalence of favorable environmental conditions for heavy infection when certain varieties happen to be at their most susceptible stage, while other varieties escape because they have not yet reached or have passed the more susceptible stage at the time of inoculation. However, it is also possible that different strains of *Venturia inaequalis* are responsible for some of the conflicting reports. The infection studies made by Wiesmann (17) as well as those of the writer show clearly that *V. inaequalis* is composed of several strains with different infection capabilities on certain apple varieties. The Yellow Transparent usually is considered relatively resistant to scab infection. The fact that this variety was entirely resistant to 7 of the 11 cultures with which it was inoculated during the present study may help to explain its freedom from scab infection in nature. The 4 cultures that did infect Yellow Transparent did so consistently and abundantly. When the 4 cultures that proved so virulent on Yellow Transparent were used to inoculate certain other varieties some of them were not able to infect at all. For example, Dudley and Missouri Pippin were entirely resistant to culture 19, which gave abundant infection on Yellow Transparent. Since most of the varieties studied by the writer were susceptible to several cultures, it seems likely that they would be found diseased in the orchard more often than a variety like Yellow Transparent, which can be infected by only certain cultures.

Much variation has been found between monoconidial isolation in their cultural characters as well as in their pathogenic capabilities on different apple varieties. However, no well-defined forms have been found that would correspond with the specialized forms of some other species of fungi. On the other hand, each monoconidial isolation of *Venturia inaequalis* made from naturally infected apples, either from the same locality or from widely separated regions, appears to differ in some of its cultural reactions and infection capabilities from any other isolation studied. Such findings indicate that *V. inaequalis* is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.

SUMMARY

Monoconidial cultures of *Venturia inaequalis*, isolated from 14 apple varieties obtained from Wisconsin, Oregon, Michigan, and New York, have been studied in relation to variability in culture, in pathogenicity, and in morphology.

Cultures differed from each other on nutrient media in the following colony characters: general appearance, margin of colony, size of colony, and abundance of conidia and perithecia produced. Cultures could easily be separated under standardized conditions by these characters.

The cultures also differed in their physiologic characters. The rate of growth has not been correlated with the locality or variety from which the cultures were isolated, since variations between isolations from the same variety and locality were as great as those between cultures from different varieties and States.

Although the optimal temperature for growth of all cultures was approximately 20° C. yet, at this temperature, certain cultures grew twice as rapidly as others.

Likewise, though the optimal pH for growth was between 4.8 and 5.8 under the conditions of these experiments for all cultures studied, with the exception of 30, yet, at this hydrogen-ion concentration, certain cultures grew much faster than others.

Culture 18 differed from cultures 21, 17, 19, 24, and 30 by better growth on solutions containing $Ca(NO_3)_2$ as the source of nitrogen than those containing KNO_3.

Cultures varied in their tolerance to different concentrations of copper sulphate and Paris green in the nutrient on which the fungus was grown.

During the 3 years that these cultures were under observation some remained quite stable, while others, at times, produced variants in the form of sectors of the parent colonies. Such sectors differed from the respective parent colonies in general appearance, production of spores, and rate of growth.

The species of Amelanchier, Aronia, Cotoneaster, Crataegus, and Sorbus studied were not infected when inoculated with 6 cultures of *Venturia inaequalis*.

Of the 13 Malus species inoculated 9 were infected by 1 or more of the 6 cultures used and the other 4 showed no disease. Differences were found in the infection capabilities of the 6 cultures on the susceptible hosts.

Each of the 20 apple varieties inoculated was infected by one or more of the 9 cultures. Fifteen varieties were infected by each of the cultures with which they were inoculated, but the other 5 varieties were resistant to some cultures and susceptible to others under greenhouse con-

ditions. The cultures differed in their infection capabilities on the Yellow Transparent, McIntosh, Dudley, Missouri Pippin, and Hubbardston Nonsuch varieties.

Morphologic differences of certain cultures were found in the length and shape of the conidia produced in culture under uniform conditions. Five of the 7 cultures had spores of approximately the same length, while the spores of the remaining 2 were of different length. Conidial length also was influenced by the variety on which the spores were produced.

This study indicates that *Venturia inaequalis* is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.

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COMMELINA NUDIFLORA, A MONOCOTYLEDONOUS HOST OF A CELERY MOSAIC IN FLORIDA

S. P. Doolittle and F. L. Wellman[1]

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During the winter of 1928 a number of celery fields at Sanford, Florida, were severely damaged by a disease that produced a brown discoloration and shrivelling of the leaf stalks accompanied by a stunting of the plants. The losses from this trouble were confined to a limited area; but, where it occurred, yields were reduced from 10 to 80 per cent. The disease was brought to the attention of the senior writer late in the spring of 1928 but the crop had then been harvested in most of the badly damaged fields, and the symptoms on the remaining diseased plants had been complicated by the presence of blackheart and recent frost injury. It seemed possible that the trouble was of the mosaic type, but, as seen under the existing conditions, diagnosis was difficult.

In the spring of 1929 the disease again developed in severe form over the same area, and, at the request of the growers, several trips were made to Sanford during the latter part of the season. Investigations at this time proved that the disease spread rapidly in the field, that it was one of the mosaic group, and that it was readily transmitted by the melon aphid, *Aphis gossypii* Glover. During the summer of 1929 some further experiments were conducted in the greenhouses of the Department of Plant Pathology of the University of Wisconsin, at Madison, Wisconsin, and in the fall and winter of 1929–1930 a detailed study of the disease was begun by the senior writer at Sanford, the results of which are included in this paper (2). Since then the work has been continued by the junior writer and is still in progress.

GEOGRAPHICAL DISTRIBUTION

The mosaic of celery, *Apium graveolens* L., here described has been found in all the important celery-producing sections of Florida and plants having apparently identical symptoms have also been found by the writers in California, Wisconsin, and Ohio and on market celery from New York. In 1922 Poole (9) described a mosaic of celery in New Jersey, and Elmer

[1] The early portion of this work was conducted at the Tomato Field Laboratory of the Florida Agricultural Experiment Station at Bradenton, Florida. Certain experiments also were conducted at the Department of Plant Pathology of the University of Wisconsin at Madison, Wisconsin. The writers are indebted to both institutions for the use of laboratory and greenhouse space in the course of this work.

The writers wish also to acknowledge the kindness of Mr. W. E. Stone and Mr. C. F. Stahl of the U. S. Bureau of Entomology in furnishing temporary office and laboratory facilities for the work at Sanford, Florida.

(4), in 1924, reported mosaic on celery in Iowa. It is probable, however, that they were dealing with a different virus. Harvey (6), in 1925, reported a mosaic on celery in Minnesota with leaf symptoms that appear to be the same as those found by the writers in Florida. No mention, however, was made of the discoloration and shrivelling of the petioles. Since 1924 the Plant Disease Reporter has contained reports of celery mosaic from States along the Atlantic Coast, as well as in Ohio, Indiana, Iowa, Minnesota, California, and Utah, but these limited reports do not permit any determination of the type of virus concerned.

LOSSES FROM MOSAIC

Celery mosaic occurred in the Sanford section for some years prior to 1928, and certain growers suffered occasional losses from the disease. In general, however, it attracted little notice. It seems to have become increasingly important in 1927 and, in the spring of 1928, caused losses from 10 to 80 per cent over an area of about 250 acres. In 1929, the damage was even more severe and some plantings were never harvested. Since the cost of production in the Sanford area is from $300 to $325 per acre, the monetary loss was great, even when the damage was confined to a comparatively limited acreage. Local estimates placed the loss at $50,000 in 1928 and at approximately $75,000 in 1929. In 1930 and 1931 the disease was much less severe; but, in 1932, although losses due to insects and fungous diseases complicated any estimate of losses from mosaic, it is safe to say that the damage was much greater than in any previous year.

SYMPTOMS

Healthy celery plants as seen in the field are of uniform height and of uniformly green color; but, when mosaic plants are present, an irregular, ragged, and readily noticeable effect is produced. The early symptoms of mosaic, as seen in Florida, consist of a pronounced outward and downward curling of the younger petioles, which gives the heart of the plant a characteristic open, flattened appearance (Fig. 1, A). The leaflets show a greenish yellowing along the veins, which later develops into whitish areas of irregular outline. The intervening green areas in the lamina remain green and this color intensifies with age (Fig. 2, A). In mature leaflets the green areas are somewhat thicker than the yellow portions. The leaflets are, therefore, slightly savoyed and crinkled. There is no filiformity or other deformity of the leaflets.

Mosaic celery plants appear stunted since the leaves tend to lose their upright habit but the length of the petioles is not greatly shortened, although their width and thickness are much diminished. As the disease pro-

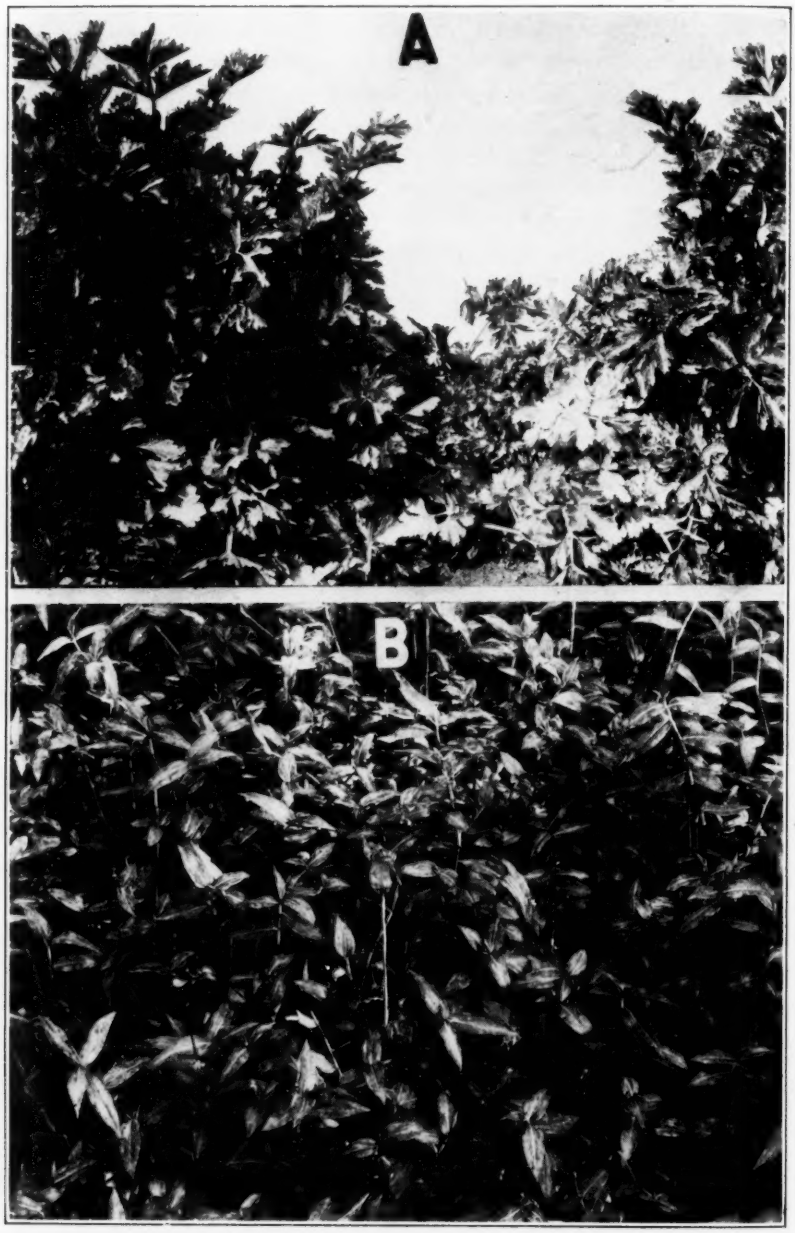

FIG. 1. A. Mosaic celery plant between healthy plants showing extreme downward curvature of petioles occurring in early stages of mosaic. B. Growth of mosaic Commelina occurring along border of celery field at Sanford, Fla.

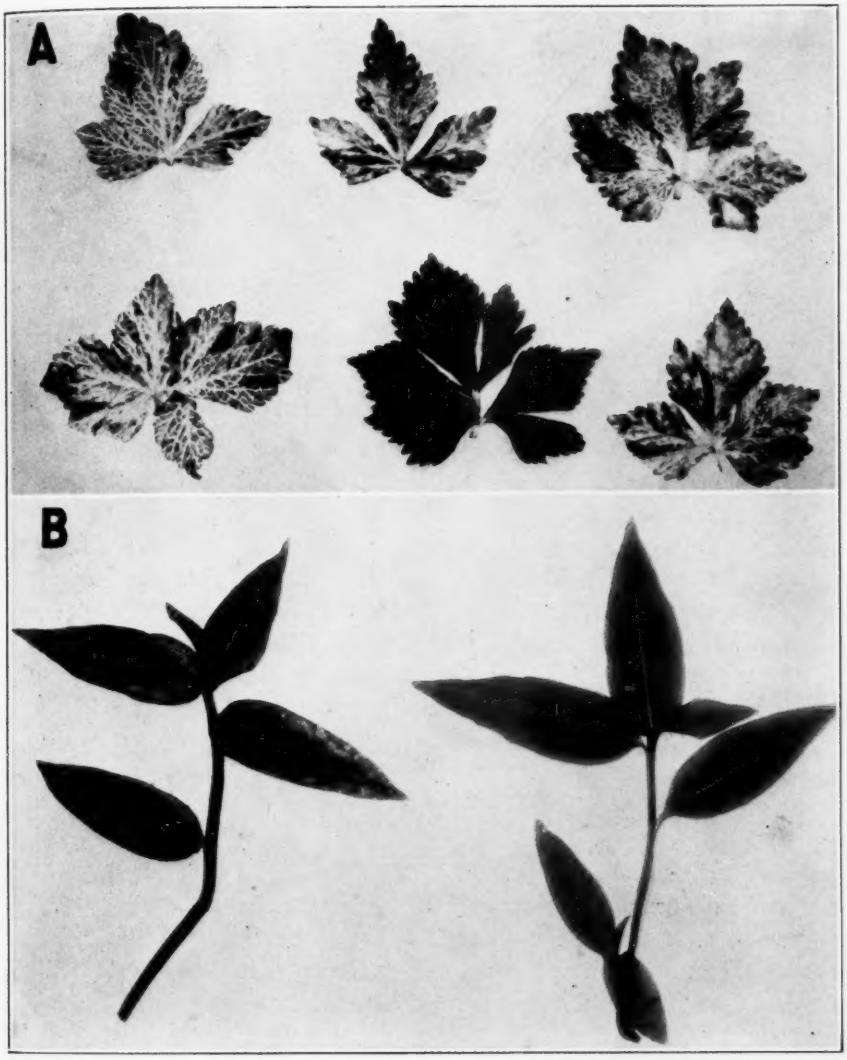

FIG. 2. A. Mosaic celery leaflets showing pronounced mottling that occurs on young leaves. Healthy leaflet in lower center. B. *Commelina nudiflora*. Plant at left shows typical mottling caused by mosaic; that at right is healthy.

gresses, buff and sunken translucent spots of irregular size and shape frequently develop on the petioles of mosaic plants. The vascular system of petioles thus affected is often discolored and, in severe cases, the petioles become brown and badly shrivelled (Fig. 3). Such plants have no commercial value.

FIG. 3. Mosaic celery plant showing discoloration, necrosis, and shrivelling of petioles. (Photographed by D. G. A. Kelbert, Florida Agricultural Experiment Station.)

Continued studies of celery mosaic, as it occurs in Florida, have made it increasingly evident that the symptoms of the mosaic we have been investigating are not those of the disease reported by Poole (9). He described mosaic plants as having a pronounced filiformity and other malformations of the leaflets, but without mottling or chlorosis. Necrosis of the petioles was not mentioned. Since these symptoms are the direct opposite of those found on mosaic plants in Florida, we, apparently, are dealing with a different virus from that described by Poole. Elmer described a celery mosaic (4) similar to that reported by Poole but mentioned a mottling of the foliage. Harvey (6), in his report of mosaic from Minnesota, figures celery leaves showing the type of mottling characteristic of the disease in Florida but he also figures a pronounced filiformity of the leaflets and makes no mention of stem necrosis. Foster and Weber (5), in 1924, were the first to describe celery mosaic in Florida, although they did not consider the petiole necrosis a symptom of mosaic.

DETERMINATION OF THE NATURE OF THE DISEASE

Aphid Inoculations

The field studies at Sanford in 1929 indicated that the disease was of the mosaic type and, since the melon aphid, *Aphis gossypii,* was abundant on celery plants in the fields where the disease occurred, the first experiments were conducted with this insect. Golden Self Blanching celery plants were secured from a section where no mosaic was known to occur and were transplanted to 5-inch pots, which were placed in a cheesecloth-covered house. After 3 weeks, the plants appeared healthy and aphids from healthy celery plants and from those showing symptoms of mosaic were transferred to plants from this stock under small cages. The aphids were transferred by means of a brush to small pieces of paper resting on a leaf of the healthy plant, and the insects soon moved to the celery foliage. Some aphids were collected from mosaic plants in the field but in most experiments aphids were taken from a known virus-free stock and colonized on mosaic celery plants that previously had been kept free from aphids. The results of these experiments, given in part in table 1, proved that the disease was readily transmitted by *A. gossypii.* A feeding period of 12 to 24 hours sufficed for the insects to become carriers of the virus and it was found that 5 aphids were able to produce infection, although 10 to 25 usually were placed on each plant. In all cases where infection occurred the symptoms were typical of those observed on naturally infected celery plants in the field.

Experiments were also conducted with the larvae of the celery leaf tyer (*Phlyctaenia rubigalis* Guenee), the garden flea hopper (*Halticus citri* Ashm.), the tarnished plant bug (*Lygus praetensis* L.), and the celery

TABLE 1.—*Transmission of celery mosaic by Aphis gossypii. (Experiments of March 13–October 2, 1929)*

Source of aphids	No. aphids per plant	No. plants inoculated	No. plants mosaic	Incubation period in days
Mosaic celery	20–35	39	38	8
" "	10	30	28	6
" "	5	12	10	8
Healthy celery (check)[a]	.....	65	0	.....

[a] Check plants received same number of aphids as used on inoculated plants.

looper (*Autographa falcifera* Kirby), but these experiments gave negative results.

Mechanical Inoculations

Attempts also were made to transmit the disease by mechanical means. In these inoculations the expressed juice of mosaic leaflets and petioles was pricked and rubbed into the leaflets and petioles of healthy plants or the freshly crushed mosaic tissue was inserted into wounds in the petioles or stem. These experiments proved that celery mosaic is transmissible by such mechanical inoculation, but the percentage of infection was much below that obtained with aphids. Table 2 shows results typical of those obtained by this method.

TABLE 2.—*Results of mechanical inoculation of celery with celery mosaic. (Experiments of April 10–October 25, 1929)*

Inoculum	Point of inoculation	No. plants inoculated	No. plants mosaic	Incubation period in days
Mosaic juice	Petiole and leaflets	36	16	10
" "	Petiole and stem	15	6	8
Mosaic-tissue fragment	Stem	15	5	8
Healthy juice or tissue (check)	As above	55	0	.....

Cross-inoculation Experiments

Since the cucumber mosaic described by Doolittle (3) and listed by Johnson (7) as "cucumber virus 1" also is transmitted by *Aphis gossypii*, cross inoculations were made by means of *A. gossypii* from mosaic celery plants to cucumber and with cucumber mosaic to celery. Infection was secured in both cases and the same results were later obtained by means of mechanical inoculations. The incubation period of the celery virus on

the cucumber was approximately that of the cucumber virus on this host, and the symptoms, particularly in the later stages of the disease, were also much the same. It was found, however, that cucumber plants affected with celery mosaic often showed a yellow bleaching of the more mature leaves; and, in many instances, the early symptoms on the young leaves took the form of small, circular yellow areas scattered irregularly over the leaf. Neither of these symptoms is usually characteristic of the cucumber virus mentioned above, but similar symptoms do occasionally occur and no marked symptomatic differences were definitely established.

In the case of celery plants affected with cucumber mosaic, the incubation period was about the same as that of the celery mosaic occurring in the field. In the early stages of the disease the celery plants infected with the cucumber-mosaic virus showed a mottling of the leaves and a discoloration of the stem not readily distinguishable from those occurring on naturally infected celery plants. When infected with the cucumber virus, however, the celery plants retained a more erect habit of growth and occasionally showed a definite filiformity of the leaflets.

In 1929 it was also shown that *Aphis gossypii* could transmit celery mosaic to the tomato, *Lycopersicum esculentum* Will., the cultivated ground cherry, *Physalis pubescens* L., and tobacco, *Nicotiana tabacum* L. On these plants the symptoms were similar to those of cucumber mosaic on the same hosts but, as in the case of the cross inoculations on celery and cucumber, there were certain differences on occasional plants. In all the comparative cross inoculations with celery and cucumber mosaic, however, the general similarity in the type of symptoms produced was so marked that there was a question as to the significance of the prevalent minor differences, particularly, since the symptoms of cucumber mosaic also varied on the same host plants. It was evident, however, that the data then available were insufficient to establish the identity of the 2 viruses. Owing to circumstances the work on this phase of the problem was temporarily discontinued until the spring of 1931 when it was again taken up by Wellman, whose studies (10) have shown that the virus of the celery mosaic here described is probably not identical with that of the cucumber-mosaic virus used in these experiments.

Sources of Primary Infection

Soil. In the Sanford section, there was a general opinion that celery mosaic first appeared at the same points in the field each season. This had led to the belief that the causal agency was soil-born and, as a consequence, caused considerable alarm. In the fall of 1929 soil was taken from about mosaic celery plants and so stored as to keep the moisture content approximately equivalent to that of field soil. Seedling celery plants, grown in a

cheesecloth cage, were transplanted to 5-inch pots of this soil immediately after its removal from the field. The experiment was repeated at intervals of 30 and 60 days. Fifty plants were used in each series and were protected from insects by a cheesecloth shelter. The plants in each series were held for periods of 40 to 60 days, but no mosaic developed. In connection with the above experiments celery seedlings of various ages were removed from the soil and the roots clipped while immersed in the expressed juice of mosaic celery plants. The plants were then immediately transplanted to 5-inch pots and grown under cheesecloth for 3 to 4 weeks. Forty-six plants were thus inoculated, but no infection occurred. It appears, therefore, that celery mosaic is not soil-borne, and field observations have definitely supported this belief.

Seed. The question of the seed transmission of celery mosaic can only be definitely settled by growing seed collected from known mosaic plants, but up to the present such seed has not been available. Tests were conducted in 1929, however, with commercial seed planted in seed beds inclosed by a cheesecloth cage. The seed used was the same as that planted in the open on an adjacent farm where mosaic had occurred regularly for several years. Approximately 2,800 plants were grown under cheesecloth, but no evidence of mosaic appeared. About 2,000 of these plants were transplanted in the field, but no mosaic developed until so late in the season that it was evident that the infection must have come from other parts of the field. Seedlings from the same seed planted in the open showed no evidence of mosaic until long after transplanting and other seed beds in the vicinity appeared mosaic-free. Observations during the seasons of 1930 and 1931 confirmed these results, and, although further work is necessary before a definite statement can be made, it is probable that seed is of little importance as a source of primary infection.

Wild Host Plants. An arrangement was made for cooperative work at Sanford with the owners of two adjacent farms where celery mosaic had been particularly severe in 1927 and 1928. Through the kindness of these growers all of the soil and seed studies and most of the work on wild hosts was conducted on these farms. In order to secure a succession of cuttings from early to late in the season, the celery on these farms was planted in successive blocks with the rows running from north to south. The plantings were made approximately 7 to 10 days apart. The investigations were started early in November, 1929, at a time when the plantings were partially completed, the last block being set out November 25.

On one of the farms the earliest celery was transplanted on October 8 in a block at the extreme north end of the farm. This area was next to the highway on the north and was bordered on the east by the buildings and

lawn of the adjacent farm. By November 15 the celery in this early planting had developed a considerable amount of mosaic, nearly all of which occurred in the first 12 rows adjacent to the farm buildings on the east boundary. In the next block, planted 10 days later, mosaic was again confined to the same rows on the extreme east side of the field but occurred only in the north end of the planting. No mosaic occurred in the southern portion of this block nor in the third planting to the south. This definite localization of the mosaic infection was manifestly suggestive of the existence of a source of infection at definite points on the east side of the field.

From north to south, the vegetation along the east edge of this field consisted first of a Bermuda-grass lawn and then a group of trees and shrubs in which there was a thick weed growth. Beyond this was a barn where weeds also were abundant. Directly south of the barn were cultivated crops other than celery and here the narrow border between the farms was clean-cultivated. The celery plants in the rows next to the lawn were almost mosaic-free but the same rows showed a large amount of mosaic beginning at the point where the field was bordered by weeds. The mosaic plants continued down these rows until the cultivated border was reached. The amount of mosaic then decreased abruptly and, after a short distance, no more mosaic plants were found throughout the remainder of the field. Since the field itself was practically weedless, this occurrence of mosaic in restricted areas along its edge was definitely indicative of the existence of weed hosts. Further observations at other points in the vicinity confirmed the fact that the disease occurred only where there was an adjacent growth of weeds.

An examination of the weeds along the border of the field in question revealed 3 species showing what appeared to be possible symptoms of mosaic. One of these, known locally as Spanish needle, *Bidens leucantha* L., showed symptoms that appeared typical of mosaic, and a large number of cross inoculations were made to celery both by mechanical inoculations and by means of *Aphis gossypii*. No infection was secured, however, and attempts to transmit the suspected mosaic to Bidens itself also failed.

A small group of wild ground-cherry plants, *Physalis lagascae* R. & S., also were found showing symptoms of mosaic, and successful inoculations were made to celery and cucumber from this host by means of aphids. The symptoms produced were those of celery mosaic, hence there is no doubt that *Physalis lagascae* may carry the celery-mosaic virus. A continued survey, however, showed that this host was extremely rare in the vicinity of Sanford and no other mosaic plants of this species were found during the season. It was evident, therefore, that its presence did not account for the general infection in the field.

The third plant showing symptoms of a possible mosaic was the creeping dayflower, *Commelina nudiflora,* L., known in the Sanford section as wild Wandering Jew. This monocotyledonous weed is common about Sanford, particularly along ditches and in moist, shaded locations, where it often forms a dense mat (Fig. 1, B). It was abundant under the trees and around the buildings on the eastern edge of the field under study but did not occur to any extent in the lawn at the north nor to the south, where the border of the field was kept cultivated. It was evident, therefore, that celery mosaic had developed only where there were Commelina plants adjacent to the rows. The plants of Commelina that were suspected of being mosaic showed no marked dwarfing of the leaves nor shortening of the internodes. The leaves were, however, distinctly mottled with areas of greenish yellow, present either as rather large, irregular longitudinal patches or as roughly circular spots from 2 to 4 mm. in diameter. These yellow areas often extended across the veins and the mottling was not confined to the sharply defined longitudinal striping that characterizes the mosaic diseases reported on most monocotyledonous hosts (Fig. 2, B). Commelina is very sensitive to low temperature, and when the first observations were made in December, 1929, the plants were making little growth and the mottling was not so marked as that observed later on the foliage produced in February and March. It was noted that, while Commelina was abundant throughout the Sanford section, the mottled plants occurred most commonly in the immediate vicinity of fields where celery mosaic occurred each year. It was also found that *Aphis gossypii* was present on occasional plants of Commelina, including some that showed symptoms of mosaic.

As a result of these observations aphids that had been colonized on healthy cucumber plants were transferred to mottled and healthy plants of Commelina growing in cheesecloth cages. The aphids fed on this host fairly readily. At the end of 24 hours they were transferred to healthy celery plants by the method previously described. All plants used for inoculation were kept under cheesecloth cages. The inoculations proved that the mottled Commelina was affected with a mosaic that was transmissible by means of aphids and the symptoms produced were identical with those occurring on celery in the field. (Table 3.)

TABLE 3.—*Transmission of mosaic from Commelina nudiflora to celery by Aphis gossypii. (Experiments of January 14 to July 31, 1930)*

Source of aphids	Number aphids per plant	Number plants inoculated	Number plants mosaic
Mosaic Commelina	15–30	25	24
Healthy " (check)	15–30	19	0

Aphid inoculations were made also from mosaic Commelina to healthy plants of the same species and infection was secured on 10 out of 15 plants. Mechanical inoculations with the expressed juices of mosaic Commelina plants were not so effective as those in which aphids were used, the results in this respect being similar to those with this mosaic on celery. Infection was secured, however, in 7 out of 32 celery plants inoculated in this manner. Reciprocal cross inoculations were made from mosaic celery plants to healthy Commelina plants by means of aphids and infection was secured in 8 out of 12 plants. Commelina mosaic also was transmitted by aphids to the cucumber and the cultivated ground cherry. In both hosts the symptoms and those resulting from infection with the virus of celery mosaic were identical, and the plants showed the same minor variations from the normal type of cucurbit-mosaic symptoms noted on these hosts when inoculated from mosaic celery plants. A mosaic on *C. nudiflora* was reported by Kunkel (8) from Hawaii, in 1921, but no description was given of the symptoms. In 1931, Cook (1) also reported a mosaic on *C. longicaulis* Jacq. whose symptoms appear identical with those described above. Neither author reported any inoculation experiments.

DISCUSSION

It is evident that *Commelina nudiflora* is a host of celery mosaic and that *Aphis gossypii* can transmit the virus from the wild to the cultivated host. The occurrence of celery mosaic on Commelina is of unusual interest, since we have what appears to be the first definitely established case of a mosaic virus affecting both dicotyledonous and monocotyledonous host plants. The mosaic on Commelina is transmissible to at least 3 families of dicotyledonous plants, and, in view of its similarity to cucurbit mosaic, it possibly may be found eventually to have a similarly large host range. In view of the fact that the mosaics of corn, cane, wheat, and other grasses appear to be nontransmissible to dicotyledonous plants, it is perhaps significant that the symptoms of mosaic on the leaves of Commelina are distinctly different in type from those reported on these hosts.

From an economic standpoint the occurrence of celery mosaic on Commelina is of great importance, since this host is abundant in Florida and its presence probably accounts for nearly all the primary infection on celery in the Sanford area. Since *Aphis gossypii* is rather common on celery and also occurs on Commelina, an abundance of insect vectors is likely to be present throughout the season. It is probable, therefore, that the eradication of Commelina in the vicinity of celery fields offers the most effective means of controlling celery mosaic in Florida, and, owing to the intensive methods of cultivation employed in the Sanford section, it is the opinion of the growers that an adequate eradication of the wild host can be accom-

plished at relatively small expense. Experiments on the control of the disease by this method are being conducted and, while still in progress, they have indicated (10) that the eradication of *Commelina nudiflora* offers a practicable and effective means of controlling celery mosaic in Florida.

SUMMARY

During the past 4 years a mosaic disease of celery has caused serious losses in Florida and has also been found in other celery-growing sections in the United States. This mosaic appears to be distinct from those mosaics described by earlier workers, since, in addition to a definite mottling of the foliage and lack of malformation of the leaflets, it produces discoloration and shrivelling of the leaf stalks.

The disease may be transmitted by mechanical inoculation and also by *Aphis gossypii*, the latter being responsible for its rapid dissemination in the field. Celery mosaic has been transmitted to cucumber, tomato, tobacco, and the cultivated ground cherry. The symptoms on these hosts and on celery are much like those of the cucurbit mosaic described by Doolittle, but there are some differences and the two viruses are not thought to be identical.

The virus of celery mosaic does not appear to persist in the soil nor in the seed, but it has been found that the disease commonly occurs on a monocotyledonous host, *Commelina nudiflora*, and in one instance it has been found on *Physalis lagascae*. Field studies have shown that the primary infection of celery can be traced directly to mosaic Commelina plants near the borders of the fields and that *Aphis gossypii* acts as the vector from the wild to the cultivated host. It is believed that the eradication of Commelina is a practicable means of control for celery mosaic under Florida conditions.

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CONTROL OF ELM LEAF SPOTS IN NURSERIES[1]

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(Accepted for publication March 11, 1933)

White elms (*Ulmus americana* L.), growing in nurseries in northern Illinois, were subjected to very severe injury by 3 kinds of leaf spot during the growing season of 1932. The fungi causing the spots were *Gnomonia ulmea* (Schw.) Thuem. (*Gloeosporium ulmeum* Miles), *Gloeosporium ulmicolum* Miles, and *Gloeosporium inconspicuum* Cavara,[2] the last being by far the most abundant and destructive in most of the nurseries seen. Accompanying the leaf spots was a severe tip blight of twigs, and when spotting was heavy severe defoliation occurred. This combination of injuries is, of course, very detrimental to the growth of young elms.

It has been suggested by Felt and Rankin[3] that "two or three applications of bordeaux at intervals of two weeks after the leaves are full grown should give reasonable control" of the elm leaf spots, but no data are available to show this to be a fact. Hence, it seems desirable to present some data obtained incidentally in 1932 while working on control measures for elm "wilt" fungi.

SYMPTOMS OF ELM LEAF SPOTS

Though the symptoms accompanying attack by the first two fungi were described by Miles,[4] the following observations seem pertinent. Leaf spots due to *Gnomonia ulmea* are first visible as small yellow to whitish flecks that develop into small, black eruptions that are limited to the upper surfaces of the leaves. These often merge into small, coal black, stroma-like structures. In many cases these stromatic structures show very distinctly because they are surrounded by a narrow band of whitish, dead tissue. Infection is not limited to the leaf. Severe damage is frequently caused also by the blighting of young twig tips.

[1] The writer wishes to acknowledge his indebtedness to Mr. L. R. Tehon, Botanist of the Illinois State Natural History Survey, for suggesting the presentation of these data and for further suggestions and assistance during preparation of this article.

[2] This determination is somewhat uncertain. The fungus involved is not identifiable as *Septogloeum profusum* (E. & E.) Sacc., *S. ulmi* (Fr.) Bri. & Cav., *S. ulmeum* Davis, or *Myxosporium roseum* Dearn. & Barth. It does, however, conform macroscopically to the fragment of *Gloeosporium inconspicuum* Cav., exemplified in No. 350 of Briosi and Cavara's *I funghi parassiti della Piante coltivate ed Utile* and gives fungus characters in agreement with the description of that species.

[3] Felt, E. P., and W. H. Rankin. Insects and diseases of ornamental trees and shrubs. 507 pp. The Macmillan Company, New York. 1932. (p. 216.)

[4] Miles, L. E. Leafspots of the elm. Bot. Gaz. **71**: 161–196. 1921.

Spots caused by *Gloeosporium ulmicolum* are visible on both the upper and lower leaf surfaces, the leaf tissue being killed in elongated spots along the midrib, veins, and leaf margins. Figure 1 shows the type of spot and the blight of growing tips caused by *Gloeosporium ulmicolum*.

The spots caused by *G. inconspicuum*, pictured in figure 2, differ considerably from the others. Characteristically, they are subcircular

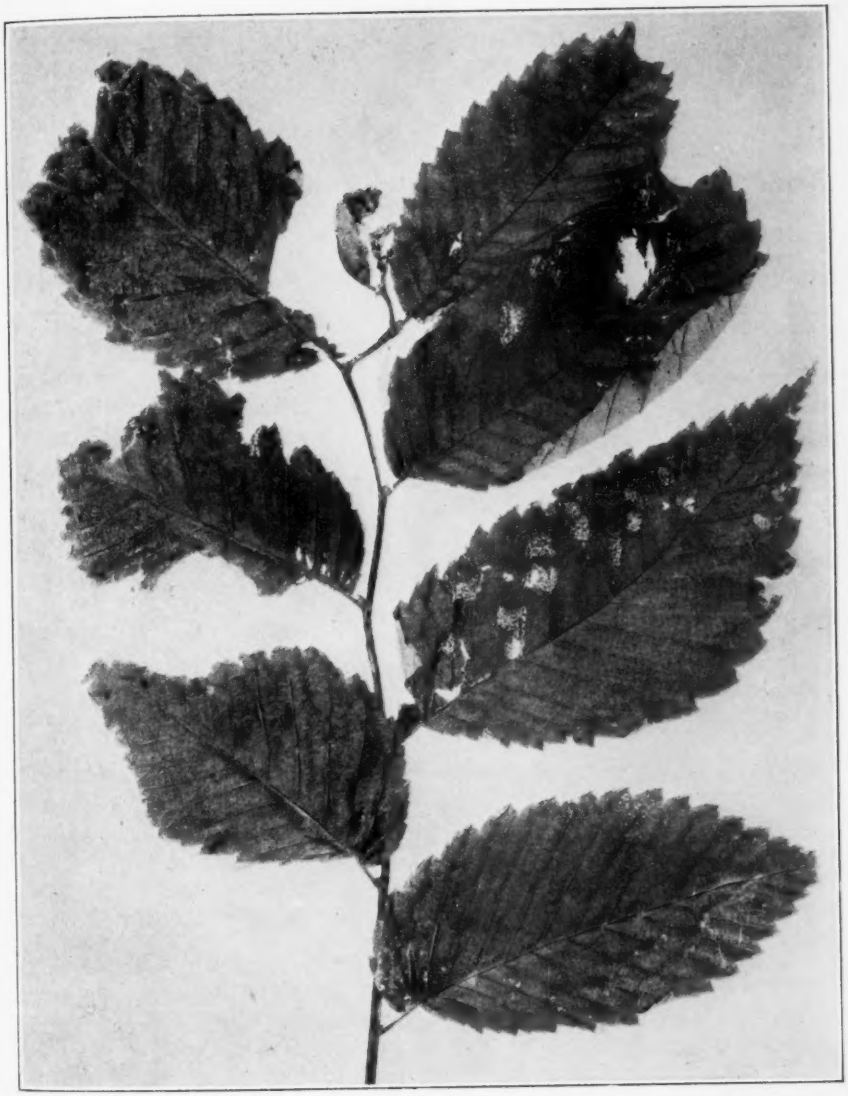

FIG. 1. The leaf spot and twig blight caused by *Gloeosporium ulmicolum*.

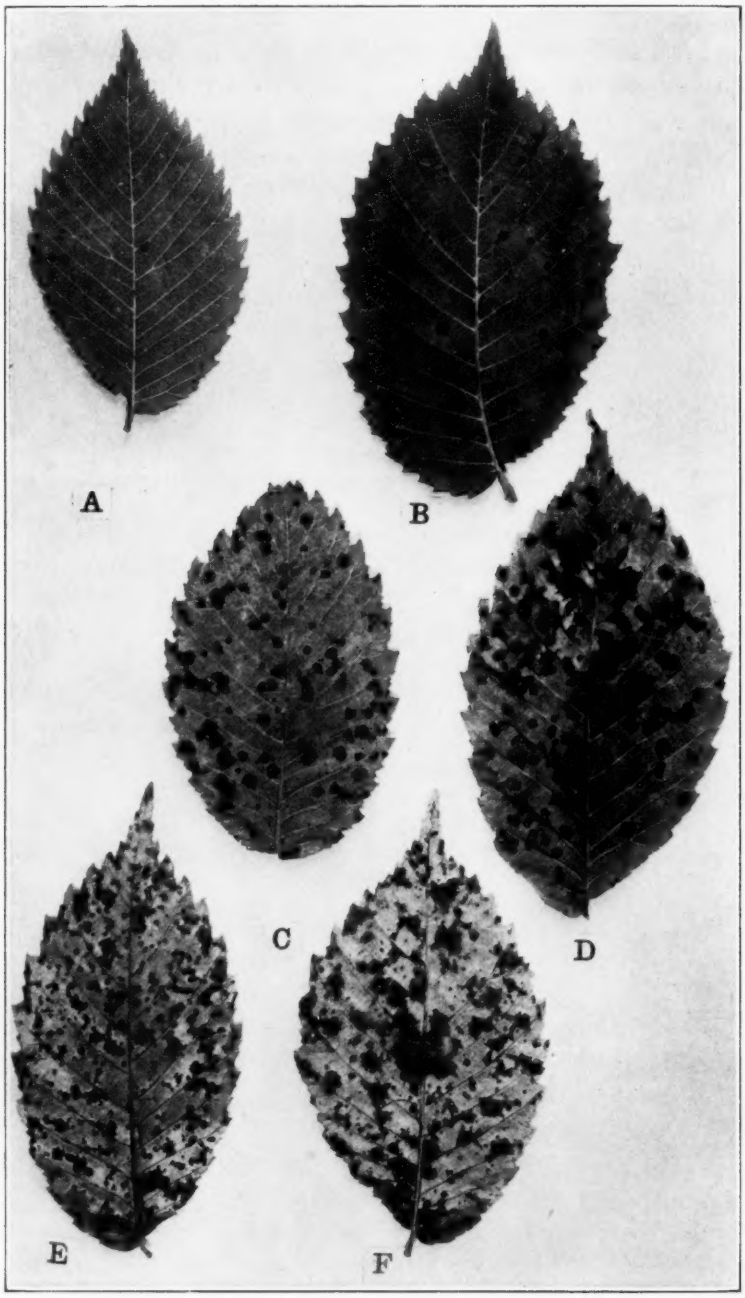

FIG. 2. Chart used as a scale in classifying leaves infected by *Glocosporium inconspicuum*. See text for method of application.

and brown, with darker margins and dark centers in which the acervuli are found. There is no development of black, stromatic tissue; and the spots may be definitely limited, effuse, confluent, or irregularly extensive along veins. They extend through the leaves and are visible on both surfaces.

Though all 3 spots were prevalent in all plots during 1932, defoliation was most severe where the *Gloeosporium inconspicuum* spot predominated, and this spot seemed generally to be more productive of early and complete defoliation than the others.

EFFECT OF WEATHER

Clinton's[5] observations on *Gnomonia ulmea* in Connecticut in 1909 led him to state: "Infection seems to take place only early in the season, since the trees early denuded did not have their second crop of leaves attacked to any extent."

In the nurseries under our observation in northern Illinois in 1932, however, both the leaf spot caused by *Gnomonia ulmea* and the leaf spots caused by the Gloeosporium species showed progressive development. Initial infection appeared about May 20, and further infection developed through June, July, and August. Progressive development was, however, more noticeable in the Gloeosporium leaf spots than the Gnomonia spot.

In the course of the season it was observed that periods of 1 to 2 weeks elapsed, when less infection occurred than at other periods. This was shown especially by trees in control plots. On them, leaves on the lowest parts of branches would be heavily infected; on branch sections just beyond, few if any spots would be found; but, farther toward the tip, leaf spot would again be heavy. This rhythm of infection probably verifies the idea that weather plays an important part, but its occurrence on treated trees may also indicate that the fungicidal applications were made too far apart. When 4 weeks intervened between applications, there was an evident tendency toward leaf-spot infection to start on the young terminal leaves. This seems to indicate that much of the infection takes place while the leaves are very young.

MATERIALS

The materials used were Bordeaux mixture, Kolodust, Koloform, flotation-sulphur dust, and dry, wettable flotation sulphur. The Bordeaux mixture used was that prepared by the Corona Chemical Division of the Pittsburgh Plate Glass Company and sold under the trade name "Corona." At 8 pounds to 50 gallons of water it gives a 4–4–50 mixture. Kolodust is

[5] Clinton, G. P. Report of the Botanist for 1909 and 1910. 1. Notes on plant diseases in Connecticut. Bienn. Rpt. Conn. Agr. Exp. Sta. 33–34; 713–774. 1910. (p. 717.)

a proprietary dust of the Niagara Sprayer and Chemical Company. It is guaranteed to contain not less than 90 per cent sulphur and is ground finely enough to permit 98 per cent to pass through a 300-mesh screen. Koloform is a wettable bentonite sulphur, also prepared by the Niagara Sprayer and Chemical Company for use as a spray, 7.5 pounds to 50 gallons of water being the proportions. The flotation-sulphur dust here referred to is prepared by the Kopper's Products Company Laboratories. The flotation sulphur used as a spray, in the proportion of 5 pounds to 100 gallons of water, is also prepared by the Kopper's Products Company Laboratories and is known as Kopper's Dry Wettable Flotation Sulphur. It is guaranteed to contain not less than 80 per cent sulphur.

METHODS

In the spring of 1932 the writer, in cooperation with several nurseries in northern Illinois, started dust and spray experiments in 10 plots, 2 of which had been sprayed twice with 3–3–50 Bordeaux in 1931. All dust applications were made with a new hand duster, but the sprays were applied with such sprayers as were available at the various nurseries. In all cases care was used to get as thorough coverage and as good applications as possible under the circumstances. The treatments given and other details are shown in table 1.

All plots consisted of normal nursery rows, or parts of rows, of American elm. They were chosen and laid out with special reference to their suitability for testing the possible prevention of infection by the so-called "elm wilt" organisms by the application of fungicidal sprays. The control of elm leaf spots was an incidental result, and only such plots are reported here as could be adequately examined.

For the present purpose plots are divided into series, according to treatment. Series A, for example, designates the part of the plot not treated, while series B and C designate sections of the plot treated with fungicides.

STANDARDS FOR MEASURING CONTROL

As it was necessary to have some means of measuring the degree of infection, an arbitrary scale was made. Leaves representing a series of 6 classes of increasingly severe infection were chosen and these were arranged on cardboard in the form of charts, as shown in figures 2 and 3, so that direct comparison could be made between them and leaves from the trees. Class A included leaves having no spots to leaves having 5 spots; class B, leaves having 6 to 60 spots; class C, leaves with 61 to 180 spots; class D, leaves with 181 to 300 spots; class E, leaves with 301 to 420 spots; and

TABLE 1.—*Details concerning nursery elm plots and treatments given them in 1932*

Plot	Series	Number of trees	Size of trees in feet	Treatments			Date of readings
				Kind	Dates given	Lbs. pressure	
II	A	450	6–10	None			
				Flotation sulphur	May 11 and 31	(Dust)	Aug. 13
	B	450	6–10	Kolodust	July 6		Aug. 13
III[a]	A	600	10–16	None			
					May 18, June 9,	200	Aug. 12
	B	600	10–16	4–4–50 Bordeaux	July 13		
IV	A	1000	5–7	None			
					May 18, June 9,	200	Aug. 11
	B	1000	5–7	Koloform	July 13		
VI	A	528	2½ inches	None			
	B	528	diameter	4–4–50 Bordeaux	May 13, June 2,	Hand pump	Aug. 25
	C	528		Wettable sulphur	July 15	" "	Aug. 25
VII	A	711	6	None			
	B	474	6	4–4–50 Bordeaux	May 13, June 2,	Hand pump	Aug. 25
	C	474	6	Wettable sulphur	July 15	" "	Aug. 25
VIII	A	800	4–5	None			
					June 16, July 8,	(Dust)	Aug. 16
	B	800	4–5	Kolodust	July 22		
IX	A	379	5–6	None			Aug. 18
		539	Yearlings	"			
	B	383	5–6	Koloform	June 15, July 9,	250	Aug. 18
		481	Yearlings	"	July 23		
X	A	400	8	None			
					June 16, July 2,	(Dust)	Aug. 10
	B	400	8	Kolodust	July 29		

[a] Sprayed with 3–3–50 Bordeaux twice in 1931.

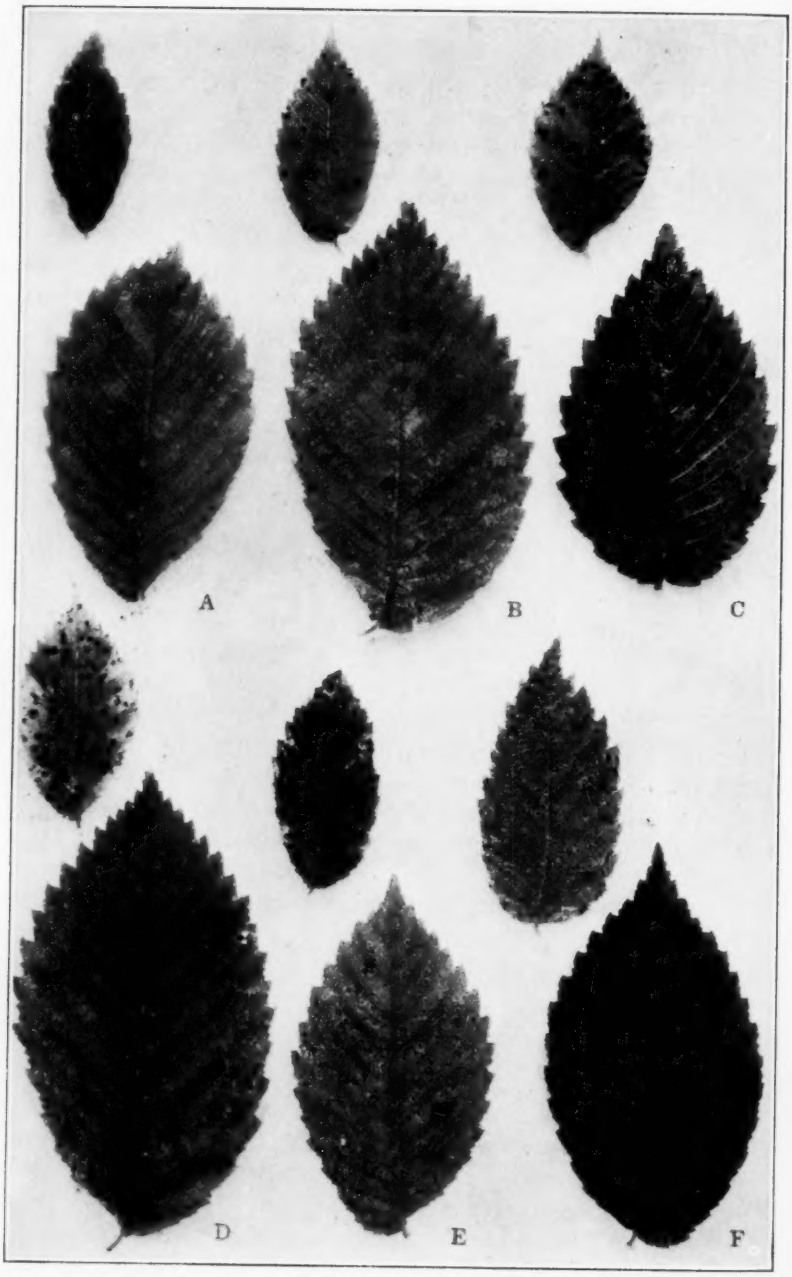

FIG. 3. Chart used as a scale in classifying leaves infected by *Gnomonia ulmea*. See text for method of application.

class F, leaves with 421 to 540 spots. These classes have the following midvalues:

A	2.5;	B	33;	C	120;
D	240;	E	360;	F	480.

By means of this scale, readings were made on every 10th tree, except in untreated series, where the occasional occurrence of trees too completely defoliated to read made it necessary to read the 9th or 11th tree. Readings were taken for all rows in plots VIII, IX, and X, and for $\frac{2}{3}$ of the rows in plots II, III, IV, and VI. In making the readings, 50 leaves from each sample tree were compared individually with the standard leaves of the chart, and each leaf was allocated according to its appearance, but without an actual counting of spots, to the class to which it seems to belong.

It will be noted that the approved method of using uniform class ranges was not followed. Although the range in severity of infection was very great, some leaves having over 500 spots, the bulk of the leaves had in nontreated series less than half that number and in treated series less than a third. A large number of small-range classes would have made the task of taking readings an impossible one in the time at hand, while uniformly large classes would have failed to show remarkable differences in control exhibited within the lowest class. The series of classes and the scales based upon them are merely practical devices, quickly designed for an immediate use.

In determining the average leaf-spot infection for a given check or treated series, the number of leaves from that particular series allocated to each class was multiplied by the midvalue of the class. The products so obtained were then summed and the total was divided by the total number of leaves examined in that particular series. The quotient so obtained represents the average number of spots per leaf for the series.

Subtracting the average number of spots per leaf for treated series from the average number in the nontreated series gives a measure of the control obtained. This measure can be translated into a percentage expressing degree of control, by dividing it by the average number of spots in the check series. By means of the percentage, different treatments in the same plot and the same treatment in different plots can be compared as to effectiveness.

DATA AND COMPARISON OF RESULTS

A summary of the readings made in each plot is presented in table 2. The differences in distribution of leaves according to the degree of infection, as between nontreated and treated series, is very apparent. In classes C to F, which represents the heavier grades of infection, the nontreated series of each plot invariably show a much greater number of leaves than the treated series. This also is true in class B, with the exception of plot IV. But in class A, which includes the noninfected and the very lightly infected leaves, the treated series invariably show a leaf number several

times as great as the untreated series. This indicates in a general way the effectiveness of the treatments in reducing infection.

A more concrete expression of the effectiveness of the several treatments used is given in table 3, in which columns 3 and 4 are summaries of the detailed data given in table 2. It is evident that the results of treatment in any one plot cannot be compared directly with the results in any other plot, because of variations in conditions between plots due to geographic separation, soil differences, situation, and the like. Nevertheless, the results obtained in each plot with one or more treatments always show an appreciable reduction in the amount of infection in favor of the fungicide. This ranges from 40 per cent in plot IX, where Koloform was used, to 90 per cent in the dry, wettable flotation-sulphur series in plot VI, and there is a general effectiveness apparent in other plots of about 75 per cent.

TABLE 2.—*Distribution of grades of leaf infection in 8 experimental plots, comparing effects of various treatments vs. no treatment*

Plot	Series	Treatment	Number of leaves per class					
			2.5 spots per leaf	33 spots per leaf	120 spots per leaf	240 spots per leaf	360 spots per leaf	480 spots per leaf
II	A	None	500	903	126	55	15	1
	B	Flotation-sulphur dust	1337	250	11	2	0	0
III	A	None	557	1227	181	27	7	1
	B	Bordeaux	1553	277	17	3	0	0
IV	A	None	50	500	449	332	131	38
	B	Koloform	910	816	108	15	1	0
VI	A	None	162	176	77	43	26	16
	B	Bordeaux	391	89	13	6	1	0
	C	Dry wettable flotation sulphur	453	35	7	4	1	0
VII	A	None	539	357	60	25	13	6
	B	Bordeaux	830	156	12	2	0	0
	C	Dry wettable flotation sulphur	874	104	18	4	0	0
VIII	A	None	1696	257	25	22	0	0
	B	Kolodust	3775	211	13	1	0	0
IX	A	None	1437	346	35	20	10	2
	B	Koloform	1788	179	24	7	2	0
X	A	None	61	930	583	300	94	22
	B	Kolodust	1328	569	79	20	3	1

TABLE 3.—*Comparison of the average leaf-spot infection in treated and nontreated series in 8 experimental plots to show the effect of various fungicides in reducing the amount of spotting.*

Plot	Series	Treatment	Number of leaves examined	Average no. of spots per leaf	Difference in favor of fungicide	Degree of control obtained[a]
II	A	None	1600	41		
	B	Flotation-sulphur dust	1600	8	33	83
III	A	None	2000	37		
	B	Bordeaux	1850	9	28	76
IV	A	None	1500	141		
	B	Koloform	1850	25	116	82
VI	A	None	500	86		
	B	Bordeaux	500	14	72	84
	C	Dry wettable flotation sulphur	500	9	77	90
VII	A	None	1000	34		
	B	Bordeaux	1000	9	25	74
	C	Dry wettable flotation sulphur	1000	9	25	74
VIII	A	None	2000	11		
	B	Kolodust	4000	4	7	65
IX	A	None	1850	15		
	B	Koloform	2000	9	6	40
X	A	None	1990	114		
	B	Kolodust	2000	19	95	83

[a] Degree of control obtained is expressed as a percentage calculated from the ratio:

$$\frac{\text{Difference in favor of fungicide (column 5)}}{\text{Average number of spots per leaf in check (column 4)}} \times 100.$$

Bordeaux mixture and dry wettable flotation sulphur, used in comparable series in plots VI and VII, both gave good control but were not consistent in both plots, Bordeaux giving 74 per cent reduction of infection in plot VII and 84 per cent in plot VI as compared with 74 per cent and 90 per cent, respectively, obtained with the sulphur. As the natural infection in the check series was nearly 3 times as heavy in plot VI as in plot VII, these results might be taken to indicate that the sulphur spray is more effective than Bordeaux under conditions that produce heavier infections. The effectiveness of a sulphur spray also is indicated in plot IV, where the heaviest check infection encountered was reduced 82 per cent by Koloform. The effectiveness of sulphur dust against both heavy and light infections is indicated, also, in plots II, VIII, and X, Kolodust, giving a reduction of

infection of 83 per cent from the heavily infected check in plot X and 65 per cent from the lightly infected check in plot VIII and flotation sulphur dust, a reduction of 83 per cent from the moderately infected check in plot II.

The results appear to justify the statement that 4–4–50 Bordeaux, dry, wettable flotation-sulphur sprays and sulphur dusts will give good control.

DISCUSSION

Trees in plots IV, series B, sprayed with Koloform and plot X, series B, dusted with Kolodust, showed considerable contrast in comparison with the unprotected trees in plots IV A, and X A, respectively, because no defoliation occurred on the treated trees, while defoliation ranged from none to as much as 75 per cent on 50 per cent of the unprotected trees. The severe defoliation in the last-named plots was due largely to closeness of planting in the nursery and poor air circulation because of size of plantings and surrounding trees.

A close correlation was observed between the length of time moisture remained on the trees during the day and the intensity of leaf-spot infection. This was noticed especially in plot VIII, which was situated on a small elevation where there was a slight movement of air, even under very calm conditions. Several times dusting was postponed in this plot until about dusk, because of lack of dampness of the foliage. Dusting was tried about sunrise a few mornings and these trees were dry, even when there was heavy dew on weeds and grass below. Leaf-spot infection was light in this plot and the effect of the dust was not so marked. In contrast, plot IV and X were located on level land between blocks of elms, which prevented much air movement. Here the dew was retained until the sun caused its evaporation late in the mornings. The moisture present made it easily possible to get a good dust coverage of the wet leaves. Likewise, the conditions were favorable for maximum leaf-spot development.

One of the advantages obtained as a result of leaf-spot control was the easier recognition of wilt. There is a very close similarity in appearance between the tips of twigs and branches defoliated by leaf-spot fungi and those defoliated as a result of wilt. Where both kinds of disease are present on the same tree, it is difficult to differentiate between their symptoms in early stages. It was, therefore, an agreeable discovery to find it possible to eliminate most of the leaf-spot infection with fungicides. Such prevention makes it more easily possible to study the symptoms and control effects on the elm wilts.

Whether or not it is practical to apply fungicides for the control of elm leaf spot will have to be determined by the individual nurseryman and will depend upon the prevalence of the fungus in his particular nursery. Control of leaf mites also may be effected to some degree with the same application of fungicides. The foliage of trees sprayed with Bordeaux or Kop-

per's dry, wettable flotation sulphur in plot VII and with Kopper's flotation sulphur in plot II was found much freer from leaf mites than the unprotected checks in these plots. The intensity of infection of elm trees with the leaf-spot fungus depends primarily upon favorable environmental conditions, since the spores are present wherever the trees are grown. Conditions conducive to an environment favorable for an epiphytotic of leaf spot are, first, sufficient rains or dew and second, trees planted close together in the rows. This environment may be amplified by having many rows together in large blocks and in a sheltered location. General observations and results of these experiments indicate that it is advisable to so modify the cultural practices in use by nurseries growing elms as to limit the size of the blocks or to alternate the tree species more often in the blocks. Especially, it seems advisable to set the trees in strips of not more than 5 or 6 rows together, leaving between these strips spaces wide enough to permit passage of a power-spray rig.

There were no indications that the fungicides used were toxic to the foliage of the elms. For trees ranging from 5 to 8 feet in height, about 1 pound of sulphur dust was found sufficient to make one application to 40 trees, while, in the case of spray material, 1 gallon gave satisfactory coverage to 5 to 17 trees, depending upon the size of the tree and the amount of foliage. For yearlings to trees 5 feet high about 1 gallon is required for every 17 trees, while trees 12 to 16 feet high require about 1 gallon for every 5 trees.

SUMMARY

Fungicides used, in 1932, in 8 experimental plots of elm-nursery stock to control wilts were found to have a very considerable additional effect in reducing infection by 3 common leaf-spot fungi, *Gnomonia ulmea*, *Gloeosporium ulmicolum*, and *Gloeosporium inconspicuum*.

The fungicides used 4–4–50 Bordeaux mixture, Koloform, dry, wettable flotation-sulphur spray, Kolodust, and flotation-sulphur dust were found to be about equally effective.

Three applications of the above fungicides reduced elm-leaf-spot infection by as much as 40 to 90 per cent, depending on the location of the plot and the amount of infection that developed.

The data presented show that 3 applications of any of these fungicides will hold leaf-spot infection under control and prevent defoliation. However, if 4 or 5 dust or spray applications could have been given over the same period of time a more constant coverage might have reduced the infection still more.

BOTANICAL SECTION,
ILLINOIS STATE NATURAL HISTORY SURVEY,
URBANA, ILLINOIS.

PHYTOPATHOLOGICAL NOTES

A Plant Inoculator.[1]—The need of a device for rapidly making uniform puncture inoculations was felt in connection with a study on resistance to bacterial wilt of corn, which required inoculation of many plants in a relatively short time. The usual method of puncture inoculation by means of an ordinary needle seemed inadequate for that purpose because progress was too slow and inoculations thus made were not uniform. Use of a common hypodermic syringe for inoculation of corn plants was found unsatisfactory because the needle plugged very frequently, the plunger was slow and inconvenient to operate, and ejection of small, uniform amounts of inoculum was difficult. The instrument here described was constructed and so operated as to make puncture inoculations easy, rapid, and uniform.

The main features of this instrument are the side opening of the needle, which prevents plugging; ejection of the inoculum by gravity; and regulation of inoculum outflow by means of an air valve. These features, previously employed in various ways, were combined in an instrument of the capacity and strength adapted to the purpose.

This plant inoculator consists of 3 major parts: needle, barrel, and air valve.

The needle is made from an ordinary hypodermic, stainless-steel, screw needle by plugging the end and making a lateral opening near the tapering end (Fig. 1, A, a). This pointed end of the needle is made very smooth and sharp to avoid cutting the plant tissue into small pieces that might occasionally cause plugging of the needle. A screw adapter (Fig. 1, A, b) is provided for attaching the needle to the barrel.

The barrel is made of a nickel-plated metal jacket (Fig. 1, A, c), with a long, wide lateral incision to permit observation of the inoculum; an inoculum cylinder of glass, inserted in the jacket (Fig. 1, A, d); and a screw cap (Fig. 1, A, e) attached to the jacket.

The air valve (Fig. 1, A, f) consists of a hollow cylindrical screw, screwed into a tube attached to the cap. A small hole is drilled through both screw and tube through which air is admitted or excluded at will.

Thick rubber washers placed at the needle end of the barrel and inside the cap make the barrel air-tight, and the glass cylinder, made slightly longer than the jacket, is pressed against the washers by screwing the cap down tight.

This inoculator was used in the following manner: It was filled with the bacterial suspension aseptically by sucking on the open-air valve and draw-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

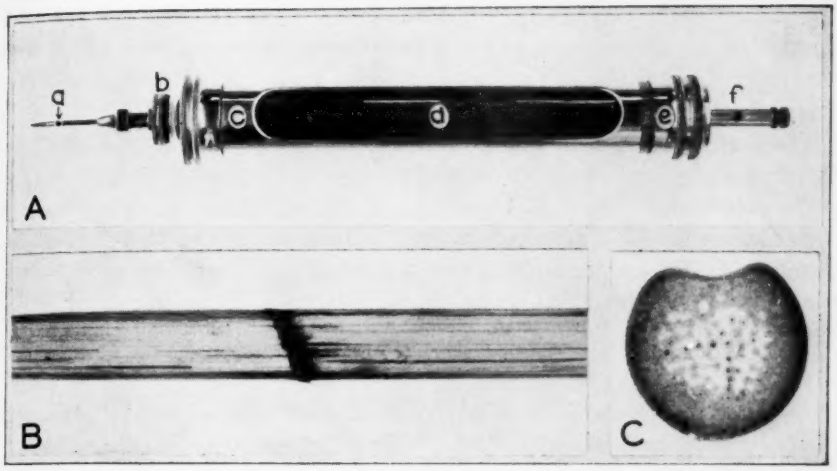

FIG. 1. A. Side view of the plant inoculator: a, hollow needle with a lateral opening; b, screw adapter; c, nickel-plated metal jacket; d, glass cylinder inserted into jacket; e, screw cap; f, air valve. B. Longitudinal section of corn stem inoculated with mixture of wilt bacteria and India ink by means of inoculator. The long, parallel black lines represent vessels filled with bacteria and India ink. The thick black line in middle represents cavity made by needle. C. Cross section of another corn stem inoculated with same mixture. Two punctures were made through stalk, one above the other at right angles. The black dots represent cut ends of vessels filled with bacteria and ink.

ing the liquid through the needle. After the inoculator was filled the hole of the air valve was closed to prevent the liquid from escaping through the needle. The filled instrument in this condition was carried from place to place in the field without spreading the inoculum. Rate of flow of the drops of inoculum through the hole of the needle was regulated as desired by adjusting the flow of air through the air valve. The most conveneint rate of flow for inoculating corn plants was 1 drop per 2 seconds.

Inoculation of a corn plant was made by thrusting the inoculator needle through the stem and withdrawing it after a second. When the tip of the needle touched the surface of the stem a drop of the inoculum ran down along the needle and was deposited at the place of contact. At the time of the withdrawal of the needle the drop was taken into the needle cavity and the vessels of the plant, probably by negative pressure and capillarity. Penetration of the inoculum into the tissue of the plant immediately after inoculation was tested in a few cases by filling the inoculator with a mixture of wilt bacteria and India ink. The presence of the India-ink particles among the bacteria facilitated tracing the path of the bacteria before and after cutting the stem open. The black suspension was observed to penetrate the vessels of the vascular tissue for more than 4 inches above and below the puncture a few seconds after inoculation (Fig. 1, B and C).

The inoculator may be made to almost any size, according to the need. In inoculating corn plants 6 weeks old or older, a 16-gauge needle ¾ in. long and a barrel of nearly 100 cc. capacity were used. More than 300 plants were inoculated at one filling.

The instrument was taken apart easily for cleaning. It was sterilized before using by boiling in water or by washing in alcohol and rinsing in sterile water.

The efficiency of this inoculator was illustrated by the fact that in the summer of 1933, 4 men, making two punctures per plant, inoculated in 4 days more than 100,000 plants.—S. S. IVANOFF, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

Losses from Potato Late Blight in 1885 and 1886.—Detailed estimates of losses from potato late blight (caused by *Phytophthora infestans* (Mont.) de Bary) for the year 1885 and 1886 have just become available to the Plant Disease Survey through the courtesy of two collaborators, S. H. Essary and C. D. Sherbakoff. The value of the material obviously warrants its publication, even though delayed. Some statement of the history of this unusual material seems also to be relevant.

The Manuscript

In February, 1933, Essary found among some old papers in the Botanical Department of the University of Tennessee a roll containing 3 manuscripts. One was a typescript of the text of Marshall Ward's paper on *Phytophthora infestans.*[1] The second was entitled "Early History of the Potato Rot in the United States" and is an excellent summary of the history of late blight in the United States, obviously taken from the reports of the Commissioner of Patents. A pencil footnote indicates that this is by E. F. Smith, and it may well have been the result of one of the first assignments of work in the Department of Agriculture to the then (1886) newly appointed assistant. The final paragraph was begun with a typewriter and concluded with pen and ink.

"Since 1850, the ups and downs of potato growing have been many. Some years, and in many sections for a series of years, there has been very little rot. In exceptional years it has prevailed almost everywhere. The last great rot period was from 1884 to 1886. *The per cent of the losses from the disease in 1885 and 1886 is presented in the following tabular statement. This table is compiled from individual reports made in reply to a circular of inquiry sent out by the Department.*"

[1] Ward, H. Marshall. Illustrations of the structure and life-history of *Phytophthora infestans,* the fungus causing the potato disease. Quart. Jour. Micros. Sci. [London] 27: 413–425. 1887.

The portion italicized was written in longhand by F. Lamson Scribner, who was at that time in charge of the Mycological Section of the Division of Botany of the United States Department of Agriculture. It obviously refers to the third manuscript, which, because of its unique historical interest, has now been deposited in the Library of the Department of Agriculture. This manuscript, as its caption indicates, contains "Tables Showing

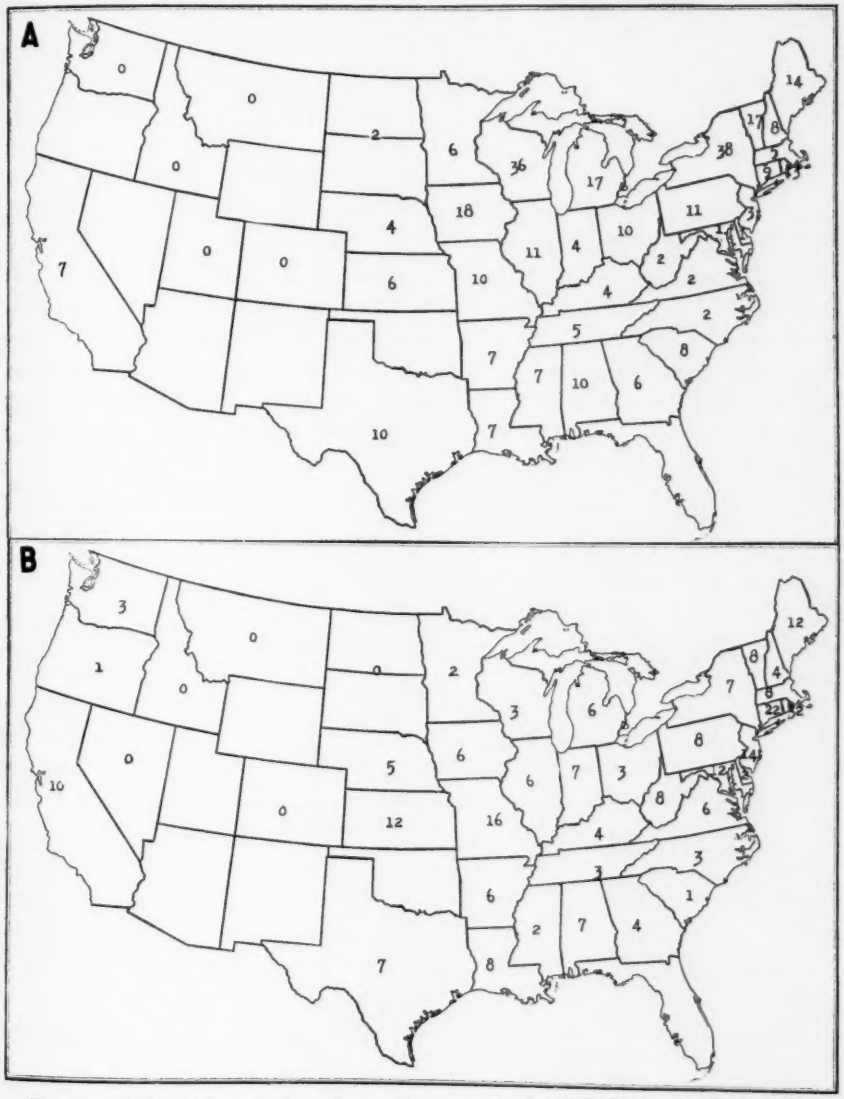

FIG. 1. Estimated percentage losses from potato late blight in the United States. A. 1885. B. 1886.

by States and Counties the Estimated Loss from Potato Rot in the United States in 1885 and 1886."

Estimated Losses

The figures on the accompanying maps, figure 1, A and B, are in the "Average Per Cent of Loss" for each State as given in the manuscript tables. They are simple averages of the estimated per cent of loss for the counties reporting. The number of reports was surprisingly large; in 1885 there were nearly 2,500 replies (page 122[2]). Michigan sent more than 200 each year and New York almost as many. Ohio, Wisconsin, and Iowa sent over 100 reports each year. These figures are admittedly mere estimates and undoubtedly estimated losses here attributed to late blight may be due in part to other causes. The distribution of the losses, however, confirms the belief of the original compilers that the chief factor in the losses was the "rot" due to *Phytophthora infestans.*

A diagram showing the estimated losses in bushels and value in 1885 for 6 States is appended to the Report of the Mycological Section for 1886.[2] The figures for the other States and those for 1886 were apparently never published. The available information can leave no doubt that in 1885 there were serious losses from potato late blight throughout the Northeastern United States. In 1886 the disease was, in general, much less severe than in 1885, but there were very large losses in Rhode Island and Connecticut.—NEIL E. STEVENS, Bureau of Plant Industry, Washington, D. C.

Pod Deformation of Mosaic-infected Peas.—In a pea-disease survey, Linford[1] in 1928 reported the occurrence of deformed pods on mosaic plants of *Pisum sativum.* In California pod damage has become of considerable concern to the grower of summer and fall peas. When the plant becomes infected with the virus prior to the completion of pod development, the pods become markedly distorted (Fig. 1, B–C). The ovary wall assumes a rough, ridged, wrinkled condition and, as a result of these corrugations, is badly deformed and somewhat dwarfed. The ridges usually appear dark green in color. Pods resulting from blossoms borne on a badly diseased vine may become so badly twisted or curled as to be hardly recognizable as pods (Fig. 1, D–F). Such pods remain severely stunted and may produce no seed. Pods may reach all conditions of maturity on mosaic plants, ranging from the aborted condition just described to that seen in figure 1, B–C, where the seeds have matured.

[2] Scribner, F. Lamson. Report of the mycological section. *In* Report of the Commissioner, U. S. Dept. Agr. **1886**: 95–138. 1887.

[1] Linford, M. B. Pea diseases in the United States in 1928. U. S. Dept. Agr. Bur. Pl. Indus. Suppl. 67. 1929.

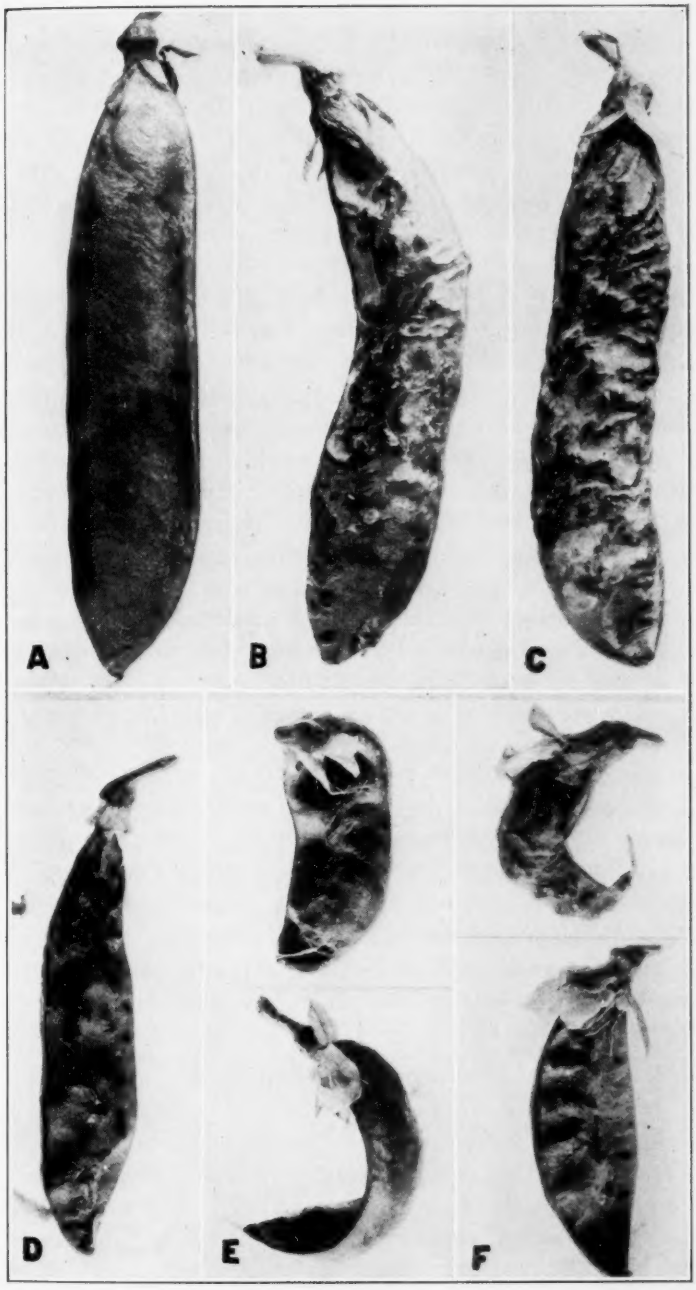

FIG. 1. Pod deformation produced by the pea-mosaic virus on *Pisum sativum*. A. Pod of a healthy plant of the Hundredfold variety. B–C. Disfiguration and roughness of pods from Hundredfold plants infected with mosaic late in their development. D–F. Badly distorted pods that have reached maximum development on vines of the Stratagem variety infected early with mosaic.

The interior of a mosaic pod also presents a roughened appearance. The knobby ridges of the exterior are replaced by creased indentations of the membranous lining of the pod cavity, and the depressions of the exterior correspond to the irregular knobby areas of the inner pod wall. The seeds in diseased pods sometimes appear smaller and yellower than normal. Upon holding an opened mosaic pod to the light, the zones responsible for the raised irregularities of the outer surface appear dark green in contrast to the green of the rest of the pod.

The foliage symptoms of pea mosaic in California agree fairly well with those Doolittle and Jones[2] described for Wisconsin; in fact, the writer obtained malformed pods at Madison in the fall of 1930 on Perfection peas inoculated with mosaic. Aphis from mosaic plants were employed in the transmission of the virus, and, under greenhouse conditions, first symptoms were expressed on the foliage after an incubation period of 6 to 9 days. In California the foliage symptoms range from a simple mottling or vein clearing of the foliage to a mottling accompanied by irregular necrotic specks or spots up to 2 or 3 mm. in extent and an extreme twisting, curling, and stunting of the growth, which, in advanced stages, becomes rosetted. The petioles, tendrils, and even stems may be distorted in very severe cases. Whether these differences are due to strains of the virus of greater or less virulence or to a virus complex, remains to be determined. At any rate, the disfigured pods occur on mosaic plants in California regardless of the particular type of leaf symptoms expressed.

As Linford noted, the loss from mosaic is sustained in two ways. First, through a decreased yield as a result of the stunting and reduced productiveness of the infected plant and second, through unmarketability of the deformed product. The latter is the most conspicuous of the two types of loss, for it becomes necessary to cull out the distorted pods on the sorting belts. The culls from this source alone amount to 10 per cent and more of the picked crop in some instances.—WILLIAM C. SNYDER, Division of Plant Pathology, University of California, Berkeley, California.

A Tropic Response in Germ Tubes of Urediospores of Puccinia graminis tritici.—During a recent microscopic examination of the surface of wheat leaves inoculated with urediospores of *Puccinia graminis tritici* Eriks. and Henn., it was noticed that many of the germ tubes of these spores grew directly across the leaves, that is, in a direction at right angles to the veins (Fig. 1). Further examination has shown that the germ tubes of urediospores have a well-marked tendency to grow transversely on wheat leaves. A brief consideration of the stomatal arrangement on the leaf will show that this

[2] Doolittle, S. P., and F. R. Jones. The mosaic disease in the garden pea and other legumes. Phytopath. 15: 763–772. 1925.

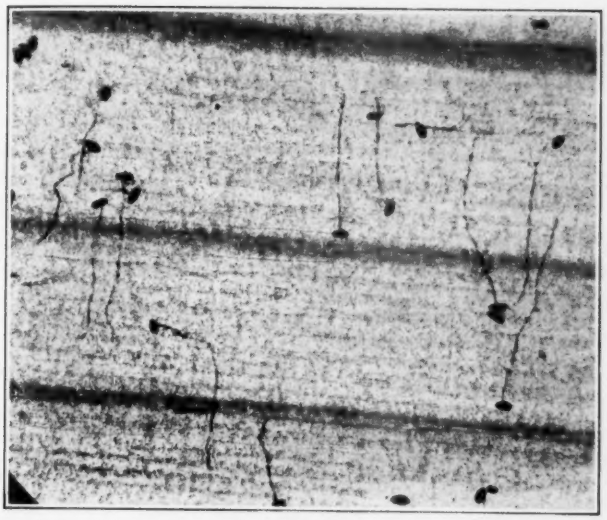

FIG. 1. Germinating urediospores of *Puccinia graminis tritici*, showing germ tubes elongating transversely on a wheat leaf.

manner of germ-tube growth is of considerable advantage to the rust as a means of securing a high proportion of penetrations. The stomata are arranged in longitudinal rows on the leaf; but the stomata of 2 adjacent rows are rarely opposite one another. Hence, a germ tube growing directly across a leaf is bound to encounter a stoma sooner or later, if it continues to elongate in a straight line.

Although this behavior of the germ tubes is known to have been observed previously by at least one other investigator,[1] no published reference to it is known to the writer. Plowright[2] and Stakman,[3] however, present drawings that show germ tubes of *P. graminis* Pers. entering stomata laterally.

In the present study a number of attempts have been made to determine the nature of the stimulus responsible for this directional growth of these germ tubes. The growth of the uredial germ tubes of *Puccinia graminis tritici* was observed on leaves of *Helianthus annuus* and a *Freesia* sp. On leaves of both species the germ tubes grew at random in all directions, except on the veins and midribs where the growth was generally straight across, as on wheat leaves. An examination of the epidermal structure of

[1] Dr. Margaret Newton, working under Prof. W. P. Fraser at McGill University, in 1919.

[2] Plowright, C. B. A monograph of the British Uredineae and Ustilagineae . . . 347 pp. K. Paul, Trench & Co., London. 1889.

[3] Stakman, E. C. A study in cereal rusts. Physiological races. Minn. Agr. Expt. Sta. Bul. 138. 1914.

the veins and midribs of these plants reveals a marked similarity to that of the wheat leaf. In both the cells are long and narrow with the lateral cell walls running parallel to one another, and at each cell wall there is a slight depression in the leaf surface. On other parts of Helianthus leaves, where the germ tubes grow at random in all directions, there is less similarity to the epidermal structure of the wheat leaf, the epidermal cells being rather irregularly arranged and the stomata more or less randomly distributed; and on Freesia leaves, the stomata are arranged in parallel rows, but the lateral cell walls are not straight as in wheat leaves but deeply sinuate.

It seems, therefore, that there is a correlation between the epidermal structure and the direction of germ-tube elongation on the leaf surface. There appears to be a well-marked tendency for germ tubes to cross cell walls at right angles. It is probable that the causal stimulus is of a thigmotropic nature, especially in view of the fact that light and geotropism were eliminated as causal agencies in the experiments. The behavior of the germ tubes was the same in diffuse light as in darkness, and the position of the leaf, whether vertical or horizontal, had no influence on the direction of growth.

There is, indeed, visible evidence of an interaction between the cell wall and the germ tube crossing it. Over each cell wall the germ tube produces a slight swelling containing an aggregation of protoplasm, as if a rudimentary appressorium had been formed by the germ tube before it crossed the cell wall. In preparations stained with cotton blue, these swellings resemble dark blue knots superimposed on the cell walls.

If the growth-directing stimulus is a thigmotropic one resulting from the contact of the germ tube with the finely grooved surface of the wheat leaf, it is apparently rather delicate, as attempts to direct the growth of germ tubes by means of artificially grooved glass, collodion, or wood surfaces have proved unsuccessful.

This apparent response to the parallel arrangement of cell walls is not confined to graminaceous rusts, as the germ tubes of urediospores of *Puccinia helianthi* (Schw.) show a marked tendency to cross the veins of sunflower leaves at right angles and to grow transversely on wheat leaves. This tendency was, however, not detected in the germ tubes of urediniospores of *Coleosporium solidaginis* Thm.—THORVALDUR JOHNSON, Dominion Rust Research Laboratory, Winnipeg, Canada.

An Improved Method of Bleaching and Clearing Leaves.—In the course of determining the detail structure of vein terminations in leaves and petals[1] it was found necessary to bleach and clear the material so that the

[1] Strain, R. W. A study of vein endings in leaves. Midland Naturalist **14**: 367–375. 1933.

epidermal cells and mesophyll were sufficiently transparent to allow study and drawings to be made of the vein endings. The reagents commonly used for this purpose did not prove entirely satisfactory. Frequently, fresh green leaves were easily cleared in a given solution, while leaves of the same species, when dry, or when collected in autumn after the chlorophyll had begun to disintegrate, were very difficult to clear or could not be cleared at all so that they could be accurately studied. Lactic acid was a reagent of this type.

Even though fresh material, as a whole, was more easily bleached and cleared than dry material, it was necessary, in order to study a larger number of species, genera, and families than were available in a living condition, to use herbarium specimens. The dried material introduced new difficulties in clearing, for many of the herbarium specimens could not be satisfactorily cleared in lactic acid, although it was heated. This resulted in the use of another substance, a mixture of lactic acid, phenol, and glycerine (Aumann's solution). This gave better results than lactic acid alone. It made the leaves firm and easy to handle, while the lactic acid rendered them soft and somewhat gelatinous. However, with this solution, many of the leaves could not be properly cleared. With continued heating in a boiling solution of this mixture, the leaves turned brown and no subsequent treatment improved their condition.

Another method was sought to clear those leaves that could not be cleared by any method used thus far. Quick and Patty[2] used, with good success, a mixture of commercial sodium hypochlorite and sodium hydroxide as a bleaching agent. This solution did not prove entirely successful with the specimens under investigation.

Since the bleaching in the method of Quick and Patty was indirectly due to the chlorine, the writer thought the introduction of nascent chlorine into water would give quicker and better results. Chlorine was prepared by the action of concentrated hydrochloric acid on potassium chlorate and used with fair results. One disadvantage was that, unless the specimens were used within a day or two, they disintegrated. It was also noted that in a few cases specimens that appeared to be bleached, turned brown when placed in the lactic acid-phenol-glycerine mixture.

The writer decided that, since alcohol is often successfully used to extract chlorophyll from leaves, the introduction of chlorine into alcohol might prove satisfactory. This was done with the best results thus far obtained. The time required for clearing the leaves varied from a few minutes to two hours, depending upon the type, size, and condition of the leaves, and the amount of chlorine introduced. Two advantages in using

[2] Quick, C. R., and F. A. Patty. A convenient method of bleaching and clearing leaves. Phytopath. 22: 925–926. 1932.

alcohol instead of water were, first, that the specimens were bleached more effectively and, second, they could be left for several days in the solution with no serious effect. It is to be understood that even with this method the leaves were afterwards heated in the lactic acid-phenol-glycerine solution to clear them completely. In all probability any leaf regardless of age or whether it is fresh or herbarium material can be cleared so well by this method that one can accurately study vein endings, epidermal or palisade cells, stomata, and crystals.—ROY W. STRAIN, University of Michigan, Ann Arbor, Mich.

A Species of Sphaceloma on Avocado.—Laboratory and field studies have shown that the Sphaceloma on avocado, *Persea americana* Mill., is an undescribed species.[1] The specific name *S. perseae* is, therefore, proposed for it and the following description given:

Sphaceloma perseae, n. sp.

Lesions generally brown to almost black, up to 3 mm. in diam.; conidial fructifications, acervuli at first, with further development constituting sporodochia or more or less free conidiophore tufts, scattered to effuse, in mass, dark olive or light brownish olive, 25–70 ± μ in length; conidiophores at first 1–2-celled, often about 12 μ high by 2–7 μ at base, tapering, or acute, to truncate at apex, arising from hyaline intra-epidermal hyphae or from prosenchymatous stroma, palisaded, on rupturing the epidermis increasing in length by continued growth or by conidia remaining *in situ* and developing as a part of the conidiophores, often 25 to 50 μ long, reaching 100 μ, more or less divergent, continuous to several septate, straight or geniculate, usually simple, sometimes denticulate, apical region often paler than rest of conidiophore; conidia acrogenous or pleurogenous, at times several produced from the same point, hyaline or colored, clear or granular, spherical to cylindrical, 2–30 × 2–5 μ; hyaline conidia ovoid or oblong-elliptical, often 5–8 × 3–4 μ, sometimes biguttulate, continuous, at least when first formed; elongate colored conidia 1–6-celled, reaching 30 × 3–5 μ, often 1–2-celled, 12–20 μ long; conidia sometimes greatly enlarged or swollen, swollen conidia at times muriform; germination by hyaline sprout conidia or by germ tubes, often produced apically or subapically but also laterally.

Maculis plerumque brunneus vel fere nigris, usque 3 mm. diam.; fructificationibus conidicis primitus acervulis, demum sporodochiis vel caespitibus plus minusve liberis, in masse obscure olivaceis vel pallide brunneo-olivaceis 25–70 μ diam.; conidiophoris primitus 1–2-cellulosis, saepe circa 12 μ altis, ad basim 2–7 μ, ad apicem attenuatis, acutisve, subobtusatis vel trun-

[1] Jenkins, A. E. The avocado-scab organism. Phytopath. 15: 807. 1925.

catis, ex hyphis hyalinis, intraepidermicalibus vel stromatibus prosenchymaticis orientibus, paliformibus, post scissum epidermidis ex conidiis in situ stantibus longioribus, saepe 25–50 μ, usque 100 μ longis, continuis vel pluriseptatis, rectis vel geniculatis, interdum ramosis; conidiis acrogenis vel pleurogenis, interdum pluribus ex singulo loco orientibus, hyalinis vel coloratis, globosis vel cylindraceis 2–30 × 2–5 μ; conidiis hyalinis saepe ovoideis vel oblongo-ellipticis, 5–8 × 3–4 μ, plerumque continuis, interdum biguttulatis; conidiis coloratis elongatis, 1–6-cellulosis, usque 30 × 3–5 μ, plerumque 1–2-cellulosis, 12–20 μ longis.

Distribution: On avocado, *Persea americana* Mill.; causing the disease known as avocado scab, United States (Florida), Cuba, Puerto Rico, Mexico, South America (Peru), Africa (Union of South Africa and Rhodesia), authority for Peru and Rhodesia based on literature reports.

Type: No. 69844, Mycological Collections, Bureau of Plant Industry; on avocado, Rockdale, Florida, Mar. 21, 1925, W. J. Krome.

Similar to *Sphaceloma fawcettii* Jenkins, but distinguished from it by the generally larger or coarser fructifications, often, in mass, light brownish olive or dark olive.—ANNA E. JENKINS, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

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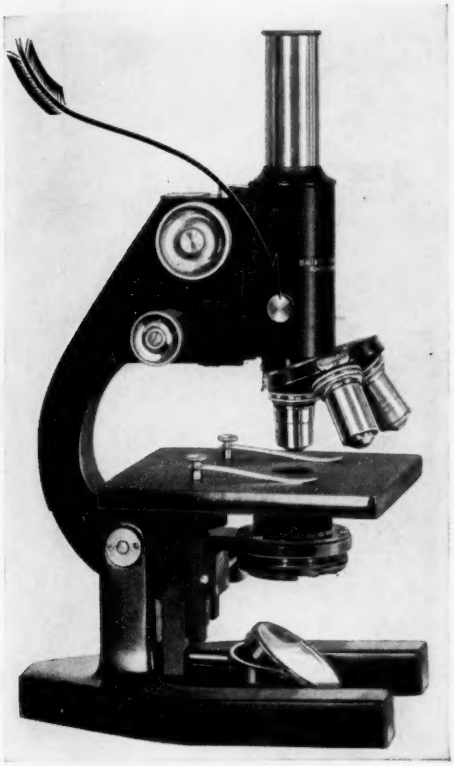

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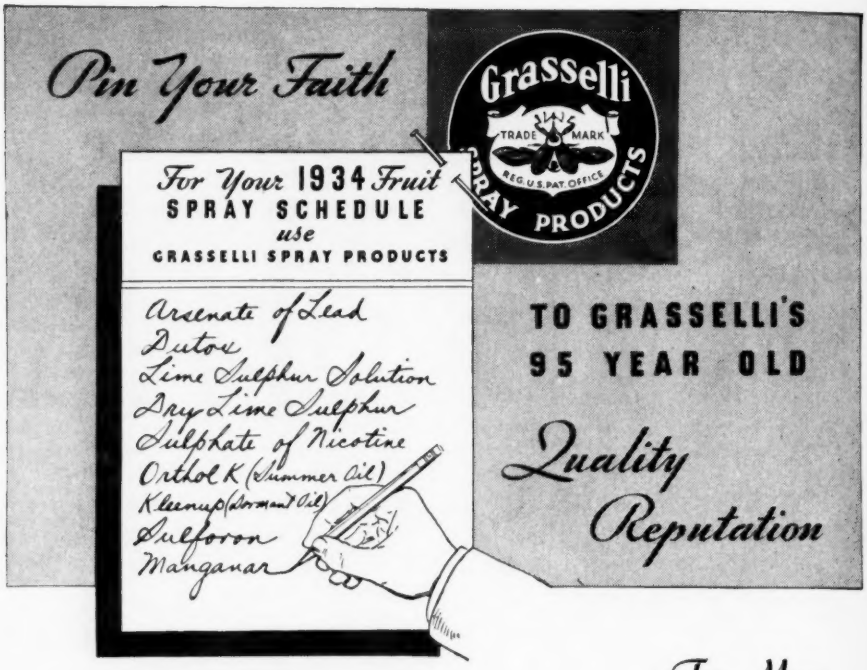

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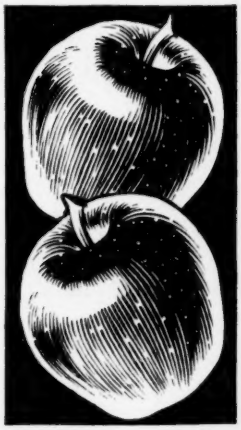

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